

Timorous plan for British cable TV

The British Government has at last decided how cable television should be regulated. Its proposals will ensure that cable will not be profitable. It should think again.

IN the course of the past twelve months or so, the British Government seems effectively to have been detached from its infatuation with the notion that the encouragement of cable television broadcasting would somehow stimulate a new industrial revolution. That is something to be grateful for, because the expectation was always more hope than reality. But the result of a year's heart-searching by the government, its advisers and its critics, is a white paper (intended as a prelude to legislation) that falls short in many important ways of the objectives the same government was loudly proclaiming at the end of 1982. And it may yet turn out that cable television in Britain, for most of the past twenty years shackled by tight restrictions only recently relaxed in thirteen experimentation regions, may in future be so shackled by restriction that it will be unprofitable, so that the "market-led" revolution that the government is still wanly hoping for will never materialize.

Technically, and with one important exception, the plan now published is sensible enough. Holding to its ambition that cable television networks should eventually become capable of two-way broad communications systems, the government has resolved to encourage the installation of cable networks with a relatively intelligent built-in topology, one in which the signals distributed to subscribers are selected (by the subscribers) from the larger menu available at crucial switching points. Such a system is obviously better suited to two-way communications systems, in which intermediate switching points in a network would be also used as ways of directing signals to their intended destinations. Whether the incentive offered to would-be cable providers — a twenty- rather than a twelve-year franchise — will be sufficient remains to be seen.

The white paper is on less certain ground in its suggestion that the maximum size of any cable television system should cover only 500,000 subscribers, that systems should ordinarily cover only 100,000, and that cable systems as thus defined should not be directly linked together but only through services provided by one of the two publicly licensed telecommunications undertakings, British Telecom and its upstart competitor called Mercury. The government is obviously worried by the prospect that some ruthless entrepreneur might choose to cover the whole of Britain with an integrated cable network — financially an entirely unrealistic danger. At this stage, and especially because Britain will not be threaded by a cable network overnight, would it not be better to wait and see what the marketplace considers sensible?

The more contentious issues raised in the past year have been neither technical nor economic but qualitative and ideological. The over-the-air broadcasting systems now operating in Britain, each of which exists because of specific legislation, are much admired and are tightly controlled. The British Broadcasting Corporation (BBC) is enjoined by its charter to be politically (and in other ways) balanced and not to offend public taste, while the goal of providing a public service is implicit in much of what it broadcasts. Commercial television (and radio) companies, on the other hand, are controlled by a statutory franchise-awarding authority whose general influence is in the same direction, but which also regulates the proportion of broadcasting time given over to advertisements. Inevitably (and understandably), both these establishments have been up in arms during the past year's debate, arguing that broadcasting standards will be undermined if

unrestricted cable is allowed. The government now sensibly rejects their arguments that pay-TV should not be allowed on the new cable systems, but proposes to set up yet another authority to license providers and operators of cable systems (not necessarily identical), and also to regulate what will be broadcast, the time given to advertisements and the proportions of foreign material that may be broadcast on cable systems — From the frying pan into the fire?

The most obvious danger in what is now proposed is that the new authority, composed though it will no doubt be of well-known men and women, will prove to be incompetent in deciding what makes commercial sense and nonsense. The present commercial regulatory authority, the Independent Broadcasting Authority, has even after two decades of experience of franchising proved recently to be curiously accident-prone in its decisions. The more subtle danger is that the new authority proposed for the cable systems will ape the paternalism of its predecessors, insisting on the application of what it considers the highest standards — but then bending the rules if its licensees run into money problems. The result will be a recipe for making sure that cable television is never more than marginally profitable and thus not the engine of a market-led development that the government has been looking for.

But what other policy could do the trick? There are several alternatives that the government has not seriously considered, including the administratively simple course of sweeping away all regulatory authorities. But an eminently practical device for saving British pride in public service broadcasting while letting cable go free would be to index-link the BBC's licence fee and to give the commercial regulatory authority responsibility for cable systems as well as old-fashioned broadcasting, doing away with the proposed restriction on the ownership of cable systems by existing commercial broadcasters. Then, at least, jealousy about shares of the advertising market would disappear, while broadcasters might find it best to use the technically most economical means of distributing their signals. □

The Development of Cable Systems and Services (Cmnd 8866, HMSO, £6.00).

Sterling and research

The decline of sterling threatens university research — and makes a mockery of planning.

THE crisis in the affairs of the largest of the British research councils, the Science and Engineering Research Council, is painful but not unprecedented (see page 4). Since the 1960s, the council has been engaged on an apparently endless guessing game about the external purchasing power of sterling, seeking to square its fixed obligations to pay in other people's currencies its subscriptions to international projects, with its obligation to the British Treasury to live within its annual budget, defined in pounds sterling. When sterling has been falling on the money markets, the council has usually had to skimp on its spending on research at British universities. On some occasions, the council has appealed to the



Treasury for help, and has been bailed out. At other times, when sterling has fluctuated upwards in its external value, the council has been able to relax, and even to put up a show of generosity in dealing with its dependants. The most spectacular windfall of this kind was that which fell into the council's lap towards the end of Sir Geoffrey Allen's spell as chairman in 1981. Much as Nelson turned his blind eye to an unwelcome signal, Sir Geoffrey and his then colleagues did not offer to return their windfall from the appreciation of sterling to the Treasury but instead spent much of it on the provision of equipment in British universities. It is a fair guess that the Treasury would be more sympathetic to the problems that have now arisen if its notoriously long memory did not include a vivid recollection of that escapade.

The financial consequence for the council and for university research at large is nevertheless absurd. Faced with the need to save more than £4 million from this year's budget, the council has no choice but to raise the funds it needs by cutting back on projects that, only a few months ago, were judged to be wise expenditures of public money. The prospect for next year is on the face of things still more serious, and it is inevitable that there should be talk of the abandonment of some expensive projects or even the closure of laboratories which ostensibly exist so as to provide central services for university research. What eventually happens will depend crucially on the newly reconstituted Advisory Board for the Research Councils, which later in the year will be expected to recommend how spending on the Department of Education and Science budget should be divided among several claimants. In principle, the board could amend the provisional allocations made last November, giving the Science and Engineering Research Council some modest help. But since, with the present government's laudable devotion to the forward budgeting of public expenditure, there is unlikely to be a larger cake to cut, the edge of the council's misfortunes could be blunted only at the expense of the other research councils, which would properly complain that forward planning had been made a mockery.

If the present health of British university research is not forever to be dependent on the money markets, better ways of regulating such problems must be hammered out. British membership of organizations such as CERN at Geneva has the character of a long-term international obligation that could no more lightly be abrogated than, say, membership of the European Community. Much the same is true of the arrangements under which the research council is paying the lion's share of the cost of the observatory at Tenerife, in partnership with Spain and the Netherlands. Could not such obligations, which the research councils are not allowed to assume without the consent of the government (including the Treasury) be regarded as unshakeable international obligations whose cost should be budgeted for in the currencies in which they must be paid? Curiously enough, the Medical Research Council, formally the British subscriber to the European Molecular Biology Organization and the European Molecular Biology Laboratory, is thus protected from currency fluctuations — no doubt because it was at the outset only a reluctant subscriber. The simple but immediate need is that subscriptions to other international organizations should be dealt with the same way. Meanwhile, the research councils most directly affected should be given authority to borrow from the commercial banks.

None of this, however, will absolve the Science and Engineering Research Council from seeming the black sheep among its fellows when the science budget is reconsidered later in the year. For although the currency fluctuations that have now embarrassed the council are outside its control, the degree to which its budget is consumed by forward obligations has become uncomfortably great. The result is that rather less than half of what the council has to spend goes on the direct support of research in universities, by means of studentships, fellowships and research grants. It would now make sense to repair this imbalance not simply as a hedge against future currency fluctuations but for the sake of using resources where they can do more good. Amalgamating observatories, or closing establishments, may be prudent as well as necessary. □

Information revolution?

The British Government may be wasting money in its sponsorship of computer technology.

If somebody should invent the wheel, it is natural that others should wish to follow suit. And where wheels are concerned, imitation is likely to be swift and immediately beneficial. The development cost will be negligible, the range of applications obvious and immediate. But none of this applies to the latter-day equivalent of the wheel, the ever faster, ever cheaper, ever more commodious computer. The simplest proof of that is the efforts now being made in half a dozen major industrialized countries to "catch up", as the saying goes, with Japan in the manufacture of the next generation of computers. In the United States, both the Pentagon and a consortium of manufacturers led by Control Data are planning to spend more than they can afford on the development of very large integrated circuits. And the British Government last week said it would spend £200 million on a programme of research and development in computer technology along the lines recommended last year by the Alvey committee, commissioned by the Department of Industry to say what should be done (see *Nature* 24 February, p.646). But even this effort, spread as it will be over several years, is probably too little to be significant. And it may divert attention from more beneficial goals.

The trouble with modern technology, as the whole world knows, is its complexity. The development of computing engines has occupied the past 200 years (if one starts with Babbage) or the past half-century (Hartree, von Neumann and Turing). It is of course astonishing that in this quickly moving field, there is still no end in sight in the process of innovation. By contrast, the technology of civil aviation seems to have reached a plateau on which startling developments have long since given way to the modest improvements of performance and economy that, in civil aeronautical engineering as in other mature fields of engineering, now constitute progress. In the circumstances, it is not unnatural that both people and governments should be excited by the potential yet to be wrung out of computer technology, and eager to enjoy their share of it. The fact that the opportunities in computers have coincided with the remarkable growth of economic productivity in Japan is simply a coincidence — neither made the other possible.

What, in these circumstances, should governments such as the British try to do? The Alvey argument is that too little research and development is at present being carried on, and that there should be a coordinated programme towards which industry would contribute a substantial share (now fixed at £150 million). The Alvey prospectus is also comprehensive — software, hardware, the investigation of applications and so on. The explicit goal is, by the spending of £350 million (large by most standards, tiny in this field), to enable a smallish industrial power to look its competitors in the eye, even those now embarking on almost identical programmes of research and development.

Can this make sense? It is right and proper that governments should look out for the interests of their major industries, taking care the while to obey the rules of international competition. The snag is that if every potential competitor spends money in the same kind of way, everybody will find the money wasted. In the peculiar circumstances of the international market in computers and their software, while the revolution being wrought is still far from complete, it is already clear that the market's suppliers will become increasingly diverse in character — and specialized. The moral, which Alvey should have seen, is that at this stage comprehensive programmes of research supported by modest funds are much less likely to succeed than more concentrated efforts towards more narrow goals. Further encouragement for successful parts of the British computer enterprise — software for example — could be more profitable. The British Government has in the past two years woken up to the invention of the computer as if it had just seen a wheel for the first time. It seems not to understand that the revolution is more complicated. □

South-East Asian toxins

Is yellow rain simply bees' natural excreta?

Washington

THE startling suggestion that "yellow rain" may simply be the excrement of bees was put forward at a meeting last week in Boston, Massachusetts. The discovery of large quantities of pollen in yellow spots found on leaves in Thailand (see *Nature* 17 March, p.200) had already prompted speculation that the yellow rain — said by the US State Department to be evidence of Soviet use of toxin weapons — may in fact be of natural origin.

The idea that bees might be implicated in the story was put forward by Professor Howard Meselson of Harvard University speaking at a meeting held last month at Tufts University, sponsored by the Institute for Foreign Policy Analysis. The spots became a focus for scientific investigations of yellow rain when they were discovered, in February 1982, in Thailand along the Kampuchean border. A team of Canadian medical investigators who were then in the area reported an outbreak of an unidentified ailment among villagers at the time the spots appeared, and analyses since carried out by the US government and other countries confirm the presence of trichothecene mycotoxins in the spots. These are the toxins that the State Department has accused the Soviet Union of manufacturing for use as a weapon in South-East Asia and Afghanistan.

The finding that the spots also contain vast amounts of pollen came to light more recently. Last summer, Australian government scientists found pollen in samples obtained in Thailand, said to have come from within Laos. The Australians concluded that the presence of pollen was so bizarre that the samples had to be "fakes", the spots having been painted on the leaves (see *Nature* 24 March, p.282).

But reports from the Canadian investigators of the widespread appearance of yellow spots in the Thailand border region, and the discovery of pollen in these samples, has made most investigators doubt that all could be deliberate fakes.

Meselson was among the first to suggest that the presence of pollen in the spots might imply a natural phenomenon at work. According to participants at the Boston meeting (which was closed to reporters), Meselson's hypothesis is based on the known habit of bees to excrete large amounts of pollen at certain times of the year. Meselson suggested that this could explain the pollen spots on leaves; if the spots were then attacked by the right species of *Fusarium* fungus, then the presence of mycotoxins could be explained as well.

Predictably, many objections were raised to Meselson's idea. According to Joan

Nowicke, a palynologist at the Smithsonian Institution who has looked at several of the samples, the spots contain pollen from many different families of plants. "It's hard to imagine that any one bee would collect this tremendous diversity of pollen", she said.

Another puzzle, according to Dr H. Bruno Schiefer of the University of Saskatchewan, is why, if it is a natural occurrence, it has only been reported in the past two years. Similarly, he questioned why there were no medical records of mycotoxin poisoning if mycotoxins are naturally produced in the region.

He added that although reports of yellow rain attacks appear to be seasonal — a point in favour of a natural explanation — the season in question is January to March, a dry period when pollen production is low.

Schiefer, a toxicologist who specializes in mycotoxins, last summer prepared a report on yellow rain for the Canadian Government after a visit to Thailand.

Schiefer ticked off several other possible explanations for the presence of pollen in the spots.

- Yellow rain contains a sticky substance;

after it falls on vegetation, wind-borne pollen adheres to the spot.

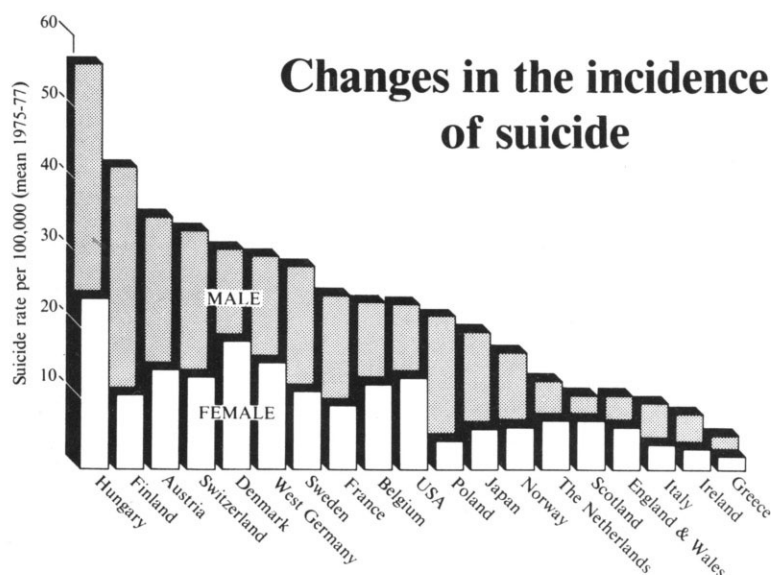
- Pollen is used in the manufacture of the toxins, either as growth medium for the fungi or — as the State Department has suggested — to provide a carrier for the toxin that could be readily inhaled.
- Pollen was mixed into the substance sprayed in the Thailand "attack" as a diversionary tactic to confuse Western investigators.

The Boston discussions, while on the one hand adding to the long list of questions about yellow rain, may also have dashed early hopes that the discovery of pollen in the samples might help to provide some answers. Nowicke explained that since it is often difficult to identify a pollen grain's family, let alone its species, it is virtually impossible to determine whether the pollen is native to the region.

Nowicke said that apart from finding a wide diversity of families in the samples she has looked at, the only other detail she could report was the size of the pollen the grains. She said that the most common size was 40 μm , although there was a large number of very small grains of roughly 10 μm . The State Department has argued that these very small grains, which are able to penetrate the lungs deeply, would be an ideal carrier for the toxins.

A report on the US government's analyses of the pollen found in its samples is expected from the Army Chemical Systems Laboratory in a month or two.

Stephen Budiansky



SUICIDE is on the increase, particularly among women and the young, according to a report published by the World Health Organization (WHO). Variations in absolute rates have done little to change the rank order of registered countries during the course of this century, although Britain has been an interesting exception to this rule, showing a 34 per cent decrease in suicide rate since 1960, as opposed to a 49

per cent increase in Hungary and a 34 per cent increase in Poland during the same period. WHO claims that this apparent discrepancy can be at least partly explained by the introduction of nontoxic natural gas supplies which made suicide by domestic gas poisoning impossible. Until 1960 this was the most common method of suicide in Britain. Source: *Changing Patterns in Suicide Behaviour*, WHO, Copenhagen.

UK Science and Engineering Council

Money fluctuations to cause cuts

THE decline of the value of sterling in the past six months has caused a crisis in the UK Science and Engineering Council (SERC). SERC pays annual subscriptions to several international research organizations, amounting to about 20 per cent of its annual budget. The decline of sterling means that the council is left with a shortfall of £4.3 million in the financial year 1983-84, which may rise to as much as £10 million a year in the future.

The British Treasury has so far refused to recompense SERC for these fluctuations. And the Prime Minister, Mrs Margaret Thatcher, has herself declined to intervene on SERC's behalf, her promise three years ago to "protect" the science budget notwithstanding. Thus the council has now to find the money from its current programmes. The brunt of the cuts is expected in the long term to fall on nuclear and particle physics, space research and geophysics.

Among others, SERC subscribes to the European Organization for Nuclear Research (CERN), the European Space Agency (ESA) and the Institut Laue Langevin, which together take up roughly 16 per cent of its annual budget (£245 million in 1983-84). SERC's immediate reaction, agreed at a stormy council meeting on 19 April, has been to cut £1 million from the budgets of each of the four grant-making research boards: the Science Board (responsible, among other disciplines, for chemistry, biology and materials science), the Engineering Board, the Nuclear Physics Board and the Astronomy, Space and Radio (ASR) Board.

In the longer term, SERC is praying that the Department of Education and Science (which is responsible for SERC) and the Treasury can find a way of making good the deficit. The Treasury has agreed to consider the problem, which is certain to be occupying the Advisory Board for the Research Councils (ABRC).

Severe cuts may, however, still be necessary, in which case the council has decided that the Nuclear Physics and ASR Boards, with the major international commitments, would suffer accordingly.

During this financial year, the council has decided that all studentships should be preserved and that the cuts should come primarily from capital expenditure, as follows:

Science Board: Improvements of the Synchrotron Radiation source at Daresbury and the development of the Spallation Neutron Source at the Rutherford-Appleton Laboratory will be delayed.

Engineering Board: New grants will be cut, even in major growth areas such as information technology and energy.

Nuclear Physics Board: The development of projects for LEP, the electron-positron

collider to be built at CERN, will probably be delayed. The Nuclear Structure Facility (a heavy-ion accelerator) at Daresbury may also suffer. How to save £1 million will be finally decided at a board meeting on 16 May. Contingency plans for the longer term have not yet been developed and the board's chairman, Professor I. Butterworth of Imperial College, London, is at a loss to know how major savings could sensibly be made. He says that the Nuclear Structure Facility has only recently been endorsed by the council after an internal review, and that its capacity and staff will already be smaller than originally planned. On the other hand, a severe cutback in the three experiments planned for LEP would, according to Professor Butterworth, be very damaging for the United Kingdom's future in international collaborations.

Astronomy, Space and Radio Board: On 25 April, the ASR Board decided virtually to halt further grant awards in 1983-84, and to cut by 5 per cent non-salary recurrent support for universities and establishments.

ASR's longer term strategy will depend on the five-year forward look to be undertaken by ABRC later this year. But the ASR Board discussed options including further cuts in university support, cuts in research within SERC establishments, major reductions in central computing

facilities and cuts in remote sensing programmes (now a growth area). Four long-term projects were earmarked for protection: the remote sensing satellite project ATSR (part of ESA's ERS-1 project), collaboration with West Germany in the X-ray satellite project Rosat, the optical telescopes at La Palma and the millimetre-wave radiotelescope due to be built at Mauna Kea.

This list leaves question marks over other areas supported by the ASR Board, such as atmospheric physics, magnetospheric physics and satellite geodesy. An attempt may be made to transfer the last of these to the Natural Environment Research Council. The EISCAT project (an upper atmosphere radar), which has suffered from technical setbacks but which is also part of an international collaboration, does not appear to be immediately threatened.

Even more radical decisions may have to be taken by the council, such as the merger of the Royal Greenwich Observatory and the Royal Observatory, Edinburgh or even the closure of an SERC establishment.

In past years, the council has benefited from currency fluctuations when they moved in the more favourable direction. The Treasury's hard line (endorsed by Mrs Thatcher) seems to be that the council must live with the swings and roundabouts. The council would, however, much prefer to be buffered or fully protected from both the benefits and penalties of currency fluctuations which are entirely outside its control.

Philip Campbell

Lawrence Livermore Laboratory

Weapons link raises hackles

Washington

EVERY five years, when the regents of the University of California decide whether to renew their stewardship of the Lawrence Livermore National Laboratory, there is a swirl of campus protest as those who deplore nuclear weapons decry their university's connection with a laboratory which tries to make them work better.

The next bout in the recurring debate was not officially due to begin until 1985 when the existing contract between the university and the Department of Energy expires. But the familiar arguments have got off to an early and unfamiliar start with an official request from Molly Lawrence, Ernest Lawrence's widow, that his name be removed from the laboratory's title. Most of the work done there, she claims in a letter to the regents, dishonours both his name and the name of a great university.

Mrs Lawrence acknowledges that her husband played a big part in the development of the atom bomb and the creation of the original radiation laboratory at Berkeley. At the end of the Second World War, she says, he believed that the existence of the bomb would prevent future wars and that atomic energy would become so cheap and plentiful that it would become

a boon to mankind. "Unfortunately his optimistic views have not been borne out. I believe if he were alive today he would be as adamantly opposed as I am to the monstrous and insane proliferation of nuclear weapons that is still taking place."

The Livermore facility was originally a subsidiary of the Berkeley radiation laboratory, but later became a separate weapons research laboratory operated by the university under a contract with the Department of Energy. Mrs Lawrence wants her husband's name to remain attached to the Lawrence Berkeley Laboratory and to the Lawrence Hall of Science but removed from the Lawrence Livermore Laboratory. She believes the existence of the two Lawrence laboratories confuses the public and also gives a wrong impression of Lawrence's attitude to his work on the bomb. It was, she says, work he did reluctantly and which he would not have wanted to mark his place in history.

Mrs Lawrence's request poses a doubly delicate problem for the regents. To defer to her wishes would risk upsetting another Lawrence family member — Ernest's brother, John, himself a regent and a scientist of conservative views who has consistently voted in favour of retaining

the link between the university and the weapons laboratory. Moreover, the decision could be implemented only with the approval of the federal government, approval which is unlikely to be forthcoming.

It is not only at Berkeley, however, that new debate is stirring on the relationship between scientific research and the development of nuclear weapons. At nearby Palo Alto, Stanford University students and faculty are waiting to hear whether the university will accept the first contract to carry out (unclassified) weapons related research at the Synchrotron Radiation Laboratory. More than 1,500 staff and students have signed a petition opposing any research explicitly dedicated to nuclear weapons development.

The administration, meanwhile, is trying to encourage scientists to increase their involvement in weapons research. Dr George Keyworth, the president's science adviser, recently chided the research community for its lukewarm response to President Reagan's call for the invention of futuristic defence systems to safeguard against nuclear attack.

Even the science adviser, however, is not above a trace of doublethink on the question of whether scientists should become involved in the defence debate. The forthcoming issue of the journal of the American Physical Society will contain a letter from Dr Keyworth which criticizes the society's decision to endorse a nuclear freeze. Political activity, the letter is believed to assert, is not a proper function of a learned society.

Peter David

Environmental pollution

Caribbean treaty promises much

THE United Nations Environment Programme (UNEP) is claiming a modest success with the signature in March in Colombia of a convention for the protection of what is called the Wider Caribbean. The convention will become effective when it has been ratified by nine of the seventeen members of the conference at which it was drawn up, and in which both Cuba and the United States participated.

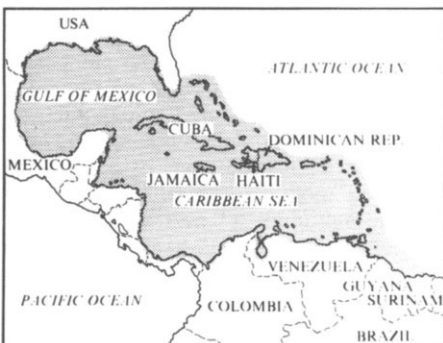
The origins of the treaty go back to 1976, when UNEP and the Economic Commission for Latin America persuaded several Caribbean states to embark on a joint environmental project. The convention signed on 24 March at Cartagena de Indias is, like those in force in the Baltic and Mediterranean, permissive rather than prescriptive. But a protocol on the handling of oil spills has been added to the convention, while another on land-based sources of pollution is being drawn up.

How quickly the treaty will be followed by a substantial degree of technical cooperation will depend on who pays how much. UNEP boasts of an action plan for which pledges of \$1.5 million are being sought. Apart from the United States, Britain, France and the Netherlands are members of the convention, on which the European Community is also represented. But Brazil, perhaps over-sensitive about the effect on the Caribbean of the outflow of the Amazon, which is carried west by the Guiana and North Equatorial currents, is conspicuous by its absence.

Dr Arsenio Rodriguez, the Mexican marine biologist now working on the project from UNEP's Geneva office, says that to begin with attempts will be made to deal primarily with coastal problems. He considers that mangrove swamps may be more sensitive than other coastal vegetation to oil pollution, and that there is an urgent need to monitor the effects of run-off pesticides on tropical marine life. The Mississippi is the most obvious but not

necessarily the most serious source of pollution, with the rapid and ill-regulated use of pesticides all round the Caribbean. There is also concern about the ease with which coral reefs — which mark the outer boundary of the wider Caribbean — may be killed, and about the disappearance of parrots and turtles from the islands.

UNEP hopes to increase its leverage by using the action fund to stimulate projects within a regional framework by national governments. Rodriguez hopes it will be possible to persuade member states to set up monitoring laboratories working to agreed standards of measurement, using a rehabilitated marine research laboratory on St Lucia as a model. Potential contributors to the trust fund have, however,



The Wider Caribbean Region, as defined by UNEP

apparently asked that nothing should be done to set up a coordinating unit in Jamaica until the work in hand is more than five times the cost of administration.

A good oil spill may be the best thing that could happen to the programme. The Ixtoc I blow-out in the Gulf of Mexico, which spilled an estimated 500,000 tonnes of oil in 1979, may indeed have spurred on the protocol on collaboration on oil spills. Now there is a prospect that the Caribbean could soon be producing 5 million barrels of oil a day.

Biotechnology

Preparing to beat newcomers

FORTIA-PHARMACIA, the Swedish-based international medical diagnostics and biotechnology company, plans to use the fat acquired during a successful 1982, when net income increased by 143 per cent, to expand research and development so as to shield itself against the small competitors springing up in the field of biotechnology.

Much of the increase of net income last year is attributable to the devaluation of the Swedish krona in October 1982. The annual report to the shareholders' meeting on 5 May shows that the group increased its net income from Skr131.2 million in 1981 to Skr318.3 million in 1982 (£1 = Skr12.05). Sales rose by 29 per cent from Skr1,441 million to Skr1,863 million, with pharmaceuticals accounting for 34 per cent of these and medical diagnostic products a further 23 per cent, but "separation products" such as chromatography kits, which made up 17 per cent of sales for 1982, will be the centre of attention for 1983. Strengthening its hold on the separation products market is intended to give Fortia-Pharmacia a substantial bite at the new biotechnology industry without taking it away from familiar ground and without exposing it to new competition.

As part of this strategy, Fortia-Pharmacia acquired three US companies during 1982 at a cost of some Skr111 million — P-L Biochemicals, the Milwaukee company specializing in fine chemicals for recombinant DNA techniques, Vespa Inc. (Pennsylvania), which manufactures raw materials for vaccines against insect allergies, and Nu Tech Medical Systems (Massachusetts), which produces catheters. The acquisitions were financed by the issue of shares in 1981.

Interestingly, the 39 per cent increase in spending on research and development to Skr207 million, or 15 per cent of sales, in 1982 seems not to have been directed towards new fields but, more conservatively, to have been channelled into the development of existing techniques such as fast protein liquid chromatography. But the company seems to be looking to cooperative agreements, such as that reached last year with the University of Uppsala to invest Skr36 million in cell biology research over the next six years.

The public offering of stock in the United States in 1981 seems to have created unexpected problems for the company. It now says that it plans to be known uniformly as Pharmacia, while the report describes the complicated arrangements being made to make intelligible to investors elsewhere the Swedish taxation law that allows a company to accumulate financial reserves without paying tax until the money is used for some business purpose.

Melanie Kee

Comecon agriculture

Hungary shows how it's done

HUNGARIAN agriculture is flourishing, much to the surprise of Soviet investigators, solely on the basis of economic incentives and guidelines. This is the gist of a report by a Soviet interministerial council set up at the end of 1981 to study economic management systems in the Soviet bloc, which has just produced its first report.

Except in Poland (where 85 per cent of agriculture remains in private hands), the standard Comecon pattern for agriculture, as for other production sectors, is one of centrally set targets and directives with which the agricultural cooperatives, collective and state farms must comply. Since Hungary has the most flourishing agriculture of the whole Comecon bloc, its success inevitably provokes heart-searching among the advocates of classical socialist central planning.

In fact, a considerable amount of low-profile planning underlies Hungary's agricultural success. Having experienced farming by directive during the Rokosi regime (1948–56), which tried, among other things, to make the country self-sufficient in such exotic products as cotton and citrus fruit, the trend of recent years has been to leave the agricultural collectives to decide what to grow but to encourage them to grow particular crops by appropriate bonuses. The scheme has not been without its blunders — in the mid-1970s, for example, bonuses were paid on the gross weight of sugar beet produced, leading to a waste of fertilizers in producing grant beet with only a low sugar content — but this was easily rectified by relating the bonuses to sugar yield.

Moreover, in the late 1970s, a detailed interdisciplinary survey was carried out with the cooperation of the Hungarian Academy of Sciences to determine the best crop for each region of the country. The survey, completed in April 1981, became the new basis for the price structure of agricultural purchases and bonuses.

To the Soviet Union, with its ever declining agricultural production, the Hungarian model must appear pragmatically tempting (although its introduction would doubtless entail some revision of Party doctrine). In a recent interview on Moscow radio, Academician Peter Vavilov, president of the All-Union Academy of Agricultural Science, blamed the Soviet Union's agricultural plight largely on "shortcomings in the functioning of the economic mechanism", although the decline in humus content of the soil, widespread erosion and a lack of capital investment and high grade fertilizers also contributed to the decline.

The main fault, according to Vavilov, was, however, the splitting up of management functions and the loss of unified control. He called for an end to "bureaucratic attitudes, parochial preoccupations, out-

moded methods of work, and inertia and laziness in economic thinking". He did not suggest that a relaxation of control on the Hungarian model should be introduced.

Indeed, the 20-year food programme, introduced in 1981 to increase food production in the Soviet Union itself, advocates a strengthening of control at every

level of the management process. So far, however, there is no sign of improvement — and 1983, the 50th anniversary of the artificial famine in Ukraine produced by the forced introduction of collectivization, is a grim anniversary for Soviet agricultural planners.

In such circumstances, their "surprise" at the success of the Hungarian system may well be gradually reflected in unpublicized but significant changes in the farm price system.

Vera Rich

Ecology groups united

Call for end to UK secrets act

A CAMPAIGN to allow greater public right of access to governmental information was launched in professional style in London last week by Mr Des Wilson, chairman of both Friends of the Earth (FoE) and the Campaign for Lead-free Air (CLEAR). FoE is currently benefiting from campaigning skills lent by Ralph Nader, the veteran American champion of citizens' rights. Nader called for civil servants and others to follow their consciences and be prepared to break the law if this was necessary to provide the public with information that would "prevent governmental and corporate crime".

The aim of the new campaign is to replace the Official Secrets Act of 1911 with a Freedom of Information Act similar to that in the United States. Seen against the backdrop of an imminent general election, the campaign has implications for Britain's major political parties courting the "green" vote. Mr Michael Foot, leader of the Labour Party, has recently made several speeches highlighting environmental issues.

Mr Wilson said that the time was not yet ripe for the formation of a new green party along the lines of the West German party. The number of votes polled by candidates standing for the existing Ecology Party in recent elections has been derisory, and FoE is instead seeking to further its aims within the major political parties. In the United Kingdom, "minority" parties are hindered by the first past the post system, and the even share of the vote gained by the German party would not give a UK green party any parliamentary seats.

Both the Labour Party and the Liberal Party say they have repeal of Section 2 of the Official Secrets Act high among their priorities for action in government. The ef-

fect of this section of the act is to make it illegal to receive almost any internal government information, so much apparently innocuous information remains classified as secret. Mr Clement Freud, Liberal MP for Ely, attempted to bring in a Private Member's Bill to reverse this position in the last days of the Labour Government. He pointed out last week that at the moment it is impossible to obtain information on which hotels have been certified as fire-worthy, for example. However, Mr John Wheeler, honorary secretary of the Conservative Party's Home Affairs Committee, described FoE's appeals for legislation leading to more open government as "incredibly naïve". He argued that in Britain it is the role of Members of Parliament to scrutinize the operation of government and claimed that comparisons with the United States were misleading. The Conservatives have no commitment to more open government at present, but their policy document for the general election has yet to be published.

The major event in FoE's 3-day publicity programme last week was the "Green Rally", which was attended by 3,000 environmentalists in London. They were in high spirits, doubtless inspired by the government's recent decision to ban lead from petrol. Speakers included Nader, Wilson, Mrs Joan Ruddock of the Campaign for Nuclear Disarmament (CND) and Regina Leshel of the German greens. CND maintains it will not be taking a party political stance in the election run-up and says there was no significance in Mrs Ruddock's sharing a platform with the Ecology Party. Mr Wilson said at the rally that the aim was simply to bring greens together in closer co-operation over the issues they shared in common.

Tim Beardsley



A gathering of greens, from West Germany, the United States and Britain.

Millimetre-wave astronomy

US could be left behind

Washington

ALARMED by the absence of plans to build any major instruments for millimetre-wavelength astronomy in the next decade, US astronomers are asking the National Science Foundation (NSF) to consider building them a new radiotelescope based on an array of millimetre-length antennas.

The telescope, expected to cost at least \$20 million, is essential if the United States is to beat off competition from Europe and Japan and maintain its lead in an area critical to the understanding of star formation and the evolution of galaxies, according to a panel led by Dr Alan Barrett of Massachusetts Institute of Technology. Without it, the panel warned the NSF astronomy advisory committee last week, millimetre-wavelength astronomy in the United States might wither away.

In asking for this kind of telescope — an aperture synthesis array — the community of millimetre-wavelength astronomers has swiftly changed horses. Until last year, when funding for the project collapsed, plans for the next decade of millimetre-wavelength astronomy rested on the construction of a single-antenna 25-metre telescope proposed by the National Radio Astronomy Observatory. The panel claims that recent advances in the reliability of receivers have made an array of antennas, producing far better angular resolution, a more suitable option.

The request has come at a propitious moment for NSF. Astronomy is likely to be awash with dollars in the next few years following the 26 per cent funding increase it received in the administration's 1984 budget proposal.

Millimetre and sub-millimetre wavelength observations have been able to make major contributions to astronomy because these wavelengths penetrate the coldest and densest interstellar clouds. They can detect many atomic and molecular transitions which serve as probes to the physical conditions within the clouds. In the next ten years, the panel predicts, such observations will be critical to research on star formation and the evolution of galaxies.

The proposed telescope would consist of an aperture synthesis array with a minimum usable wavelength of 1 mm, an angular resolution of one arc-second or better at a wavelength of 2.6 mm and a total geometrical collecting area of 1,000 to 2,000 square metres. For the time being, the panel is asking NSF only for a design study; construction would not begin for two years and the instrument would not begin to operate much before the end of the decade.

Approval of the panel's request cannot be regarded as a foregone conclusion, however. NSF director Ed Knapp told the astronomy advisory committee last week that astronomy's spectacular success in the

1984 budget apportionment had come about partly because of the astronomers' careful choice of priorities. NSF can be expected to look carefully at the possibility of US astronomers sharing international facilities.

The millimetre-wavelength panel maintains that no such facilities come near to meeting their requirements. In sub-millimetre astronomy, the future looks fairly bright, with new telescopes planned by Caltech and by Arizona University in association with the Max Planck Institute.

Agricultural research

Australia woos developing countries

Canberra

AUSTRALIA is embarking upon its strongest commitment yet to long-term aid in agricultural research for developing countries. The Australian Centre for International Agricultural Research (ACIAR), established in June last year as a statutory body responsible to the Minister for Foreign Affairs, has been given free rein for the next 10 years, after which its operations will be reviewed, with A\$25 million in the kitty for its first three years. Its funds are carved out of the foreign aid appropriation.

ACIAR's main role is to match research projects going on in institutions such as CSIRO, universities and state agricultural departments with the needs of developing countries. Beneficiaries will be, in the first instance, Australia's neighbours — South-East Asia and the South Pacific islands, particularly Papua New Guinea with which Australia has historical links.

The need for such an umbrella organization to coordinate agricultural aid, previously administered in an *ad hoc* fashion, was perceived by a study committee chaired by Sir John Crawford, chancellor of the Australian National University, and its inception was discussed as part of the North-South dialogue at the Commonwealth heads of government meeting in October 1981.

According to the then Minister for Foreign Affairs, Mr Anthony Street, when introducing the ACIAR Act, its charter is "to mobilize and expand Australia's research capacity so as to assist developing countries" and "not itself engage in research activities". ACIAR's first director, Professor R.J. McWilliam, stresses that it will not make grants and will not respond to individual applications. Rather, it will monitor research programmes in Australian institutions, supporting them only if they meet ACIAR's stringent criteria.

Among the first projects approved are studies aimed at the development of high-yielding cultivars of the pigeon pea, a crop particularly suited to semi-arid environ-

The Caltech project is still dependent on NSF funding approval; the Arizona telescope has already been promised state funds and money from the German Government. In millimetre-wavelength astronomy there are no suitable telescopes on the drawing board at all.

Some of the scientific problems confronting millimetre-wavelength astronomers could be tackled by using the Nobeyama Radio Observatory in Japan or the Institut de Radio Astronomie Millimétrique in Spain, the panel believes. But it says neither of these facilities offer the right combination of wavelength coverage, sensitivity and speed.

Peter David

ments in Asia, the development of a computer-based system to assist in the identification of virus diseases of plants, research into the use of rice straw as an animal feed in India and a series of projects on the storage of grain in the tropics, to be carried out in the Philippines, Malaysia and other South-East Asian countries. Further programmes are being prepared in biological nitrogen fixation, post-harvest technology of fruit, vegetables and fish, wheat genetics and pathology and farming systems in the South Pacific and Africa.

Vimala Sarma



Glass still around

LONDON's Science Museum this week puts on show four rare pieces of early chemical glassware. They were used by Arabs some time between the tenth and twelfth centuries for distillation and sublimation. The surface of the glass is weathered, giving a multi-coloured effect. Also on view in the exhibition which opens to the public on 5 May is new archaeological evidence. The earliest use of distillation techniques. □

Israeli science prizes

Acceptance bestowed at last

Rehovot

THE Wolf Foundation prizes, which will be presented next week in Jerusalem for the fifth time, have now come in from the cold.

Their very creation was resisted tooth and nail by the Israeli scientific establishment, which thought that the \$10 million set aside by a then anonymous donor would be better spent on financing research than in giving out prizes. Moreover, critics argued that it would be pretentious for Israel to serve as a venue for the distribution of "imitation Nobel prizes".

So in the mid-1970s, when the idea was first mooted, the heads of the Israel Academy of Sciences and Humanities pleaded with Prime Minister Golda Meir, Finance Minister Pinchas Sapir, Education Minister Aharon Yadlin and the donor that the project be shelved. The government leaders were willing to listen, but not the donor, and since it was his money the prizes came into existence.

That donor, whose identity was only made public in 1978, was Dr Ricardo Wolf, a German-born inventor who spent most of his life in Cuba before coming to Israel in 1961 as Cuban Ambassador. Although a millionaire, thanks to his development of a process for recovering iron from the residue of smelting, Dr Wolf was a great believer in social justice and an early supporter of Fidel Castro in his revolt against the Batista regime. After Castro came to power, he appointed Wolf as an ambassador, first to Italy and then to Israel.

When Cuba broke off diplomatic relations with Israel in the wake of the Yom Kippur war, Wolf, then already in his 80s, decided to remain here and devote himself to philanthropy, with special emphasis on the promotion of science and the arts. As a result he established the Wolf Foundation and brought about the passage of a special law by Israel's Knesset in 1975 whereby the five \$100,000 Wolf prizes were to be presented each year by the President of Israel in the Knesset building.

Even now, the president of the Israel Academy of Sciences and Humanities, Professor Ephraim Orbach, and his immediate predecessor, Professor Aryeh Dvoretzky, regret that Dr Wolf's gift is not being used to support the work of young scientists rather than for prizes to established scientists who, in Dvoretzky's view, "don't need the money anyway". But both Orbach and Dvoretzky are pleased that the Wolf Fund is financing some scholarships and research, and neither question the stature of those given Wolf prizes.

This is particularly clear in the field of medicine, where three of the eleven men honoured between 1978 and 1981 — Professor George D. Snell of the Jackson Laboratories in Bar Harbor, Maine, Professor Jean Dausset of the St Louis Hospital in Paris and Professor Roger W.

Sperry of California Institute of Technology — went on to receive Nobel prizes.

Medicine is also the only scientific field in which an Israeli, Professor Leo Sachs of the Weizmann Institute, has won a Wolf prize. Since that time, cancer researcher Sachs has garnered other honours, including, last month, the Bristol-Myers prize in New York.

Three "non-Nobel" fields are recog-

nized by the Wolf Foundation: agriculture, mathematics and the arts. Professor Isaac Harpaz, former dean of the Hebrew University's faculty of agriculture, says that agricultural researchers in Israel and abroad are very grateful that their work has been recognized in this way, for the first time anywhere.

Harpaz believes that Israel's prestige in the world of science is enhanced by the fact that the prizes are awarded here. This view is echoed by Professor Sachs, who says they reflect "Israeli — and indeed Jewish — appreciation of intellectual values".

Nechemia Meyers

India's space programme

Hopes soar with successful launch

INDIA has demonstrated its continuing progress in space research by putting into orbit yet another satellite using the indigenous satellite launch vehicle SLV-3. The four-stage solid-fuel rocket blasted off without a hitch from the Sriharikota launch-pad on the east coast on 17 April, placing the 41.5 kg satellite in an elliptical orbit at 45 degrees inclination with perigee 438 km and apogee 975 km. The Rohini-II (named after the birth star of Lord Krishna, the Hindu god) is second in the series of "50-kg" satellites India has been building with Soviet hardware and technical assistance. The latest satellite carries a charge-coupled device array imaging system called SMART to be evaluated for remote sensing applications.

The successful launch has restored the morale of the Indian Space Research Organization (ISRO), which had reached a low point after the failure of the US-built multipurpose commercial satellite (INSAT-1A) which followed an unsuccessful SLV-3 launch. But the orbiting of Rohini-II has changed all that and Prime Minister Mrs Indira Gandhi, who witnessed the latest SLV-3 launch, promised more funds for space research in the next ten years.

SLV-3, intended to be the workhorse of India's future space missions, had been flight-tested three times previously but only once successfully. ISRO now says there will be no more developmental flights and SLV-3 is considered operational. Its future payloads will be applications satellites, including one for reconnaissance. The Indian planning commission has already sanctioned funds for an augmented SLV (ASLV) that will have two strap-on solid boosters to give 150 kg payload capacity. ISRO has also been given the go ahead for the ambitious polar satellite launch vehicle (PSLV) project aimed at placing 1,000 kg satellites in polar orbit.

PSLV will use a liquid-fuelled engine in the second stage identical to the Viking engine used in the Ariane launcher of the European Space Agency. The first PSLV launch is scheduled for 1986 and work on testing the liquid propulsion system is soon to start at the newly acquired site on 5,000

acres of forest land near Trivandrum.

Meanwhile ISRO is getting ready for the INSAT-1B multipurpose satellite scheduled for launch by the US space shuttle in August. INSAT-1A and INSAT-1B were built by the Ford Aerospace Corporation, with whom negotiations have begun for INSAT-1C. □

Exosat — please wait

THE European Space Agency (ESA) has now fixed a date for the launch of the X-ray satellite Exosat — but once more the astronomical community is trying to persuade it to change its mind. The scheduled launch date of 26 May will give the satellite a useful lifetime of about 2.8 years. But the astronomers say that by delaying the launch by a month, the useful lifetime could be extended by a year. Unfortunately, the change would involve a financial penalty for ESA — and some loss of face.

The Exosat satellite has had a troubled time ever since its conception, most recently because of ESA's intention to launch the satellite on the delayed Ariane rocket. Earlier this year it was agreed (see *Nature* 3 March, p.4) that the satellite should after all be launched on a Thor-Delta rocket from the Vandenberg test range in California, and ESA's director of scientific programmes, E. Trendelenburg, duly signed a contract with the National Aeronautics and Space Administration (NASA) for a launch on 26 May.

Unfortunately, it has since become clear that a later launch would permit Exosat to use an orbit that combines a greater perigee height (and hence less air resistance) with more efficient use of fuels to control spacecraft attitude, increasing the useful lifetime to four years. The useful lifetime increases quite rapidly with launch delay, but at a diminishing rate so that even a few days could make a substantial difference. The question now is whether ESA can be persuaded by Exosat's scientists to pay the money required both internally (to keep the ESA Exosat team together) and externally (to reimburse NASA for the extra costs involved).

Philip Campbell

Disarmament plea

Anti-missiles not cricket

THE banning of the US anti-missile system recently announced by President Reagan has been called for by 244 Soviet scientists and academics, including officials of the All-Union and Republic Academies of Sciences. Although this is not the first time that such an appeal has been made by Soviet scientists, in the past such documents have dealt with a particular sector of warfare (nuclear or chemical). The Soviet Union and its Warsaw Pact allies now contend, however, that President Reagan's proposed system effectively adds a new sector to the arms race, by strengthening first strike capacity to a point where the United States could win a nuclear war by a preemptive strike, without necessarily risking its own destruction.

The scientists' document does not spell out the situation so plainly. It simply claims that President Reagan's proposal to create "defensive weapons" is simply a bluff. Such a weapon, it says, can do "virtually nothing" for a country subjected to "sudden massive attack", and the President simply wants "destabilization of the existing strategic balance". It draws attention to the "authoritative and responsible statement" already signed by representatives of the academies of sciences of many countries, including the Royal Society (United Kingdom) and the Académie Française, supporting the view that there is "no effective means of defence against nuclear war".

One of the signatories, Dr Georgii Arbatov, director of the Institute of the United States and Canada of the Soviet Academy of Sciences and widely regarded as an unofficial spokesman for the Kremlin on North American matters, said on the English language service of Moscow radio that the proposed American "defensive" system "absolutely derails the whole process of arms control" and that, furthermore, the system, based on computer response, would be too prone to technical failure, which could have tragic consequences. Moreover, he warned, although the American President might suggest that, once a fool-proof anti-missile system is worked out, the Americans would share it with the Soviets, the Soviet Union did not intend to "wait twenty or thirty years for American generosity". He appeared to be echoing the words of General Secretary Yuri Andropov, who last month warned that "all attempts to achieve military supremacy over the Soviet Union are in vain".

In spite of Mr Andropov's warning, Soviet politicians and columnists continue to urge the need for arms limitation. The 56 official slogans for this year's May Day celebrations include two appeals to the peoples of the world to halt the arms race and to prevent a nuclear catastrophe, and a call to the people of Europe not to allow the

deployment of new American missiles in Western Europe.

In the celebrations of Cosmonautics Day (12 April), Cosmonaut Shatalov reiterated Andropov's commitment to resisting the attempts of any foreign power to achieve military supremacy, said that the Soviet Union has "always urged and continues to urge" that space initiatives should be "exclusively peaceful". (This is, on paper, true; in spite of Western evidence to the contrary, the Soviet Union has always denied allegations of involvement in a space-weapons programme, hunter-killer satellites, beam-weapons and the like — although the best Western estimates put the military contribution to Soviet space funding at around 60 per cent.)

Forecasting that within the next ten years the Soviet Union would maintain permanently staffed space-stations on a shift system "just as we now send various scientists to the Antarctic", Shatalov called for all work in space to be "directed towards the more rational utilization of natural resources of our Earth, and to help people on Earth live still better". **Vera Rich**



PROFESSOR Richard Norman, FRS, of York University, is to be Chief Scientific Adviser to the Ministry of Defence from September. As such he will be responsible for advising the Secretary of State for Defence, the Rt. Hon. Michael Heseltine, on all scientific aspects of defence.

Professor Norman, aged 51, has held the Chair in Chemistry at York since 1965. He has worked on applications of electron spin resonance spectroscopy in elucidating structures of organic radicals and mechanisms of reactions, and is author of two textbooks on organic chemistry.

The post is normally held for 5 years. Professor Norman said last week that he was looking forward to an "enormously challenging job" in which he would deal with the entire spectrum of science. He succeeds Professor Sir Ronald Mason, also a chemist, who has returned to the University of Sussex.

Tim Beardsley

French universities

Protests hit their targets

THE Government of France backed away at the weekend from the most rigorous interpretation of its intentions for higher education. Riotous demonstrations last week by students in Paris seem painfully to have reminded ministers of the upheavals of 1968. And the prospect of strikes planned for this week by the hospital doctors prompted the Prime Minister, M. Pierre Mauroy, to say on 29 April that the new regulations affecting medical students would not take effect until the academic year beginning in 1984.

The political future of the Minister of Education, M. Alain Savary, now the sole architect of this further round of university reform, is now under a cloud, his defensive interview with *Le Monde* on 30 April notwithstanding.

The issue underlying the events of the past three months is the government's ambition to limit access to the successive stages of higher education in France. Medical education has been particularly affected by a decree, issued last December, by means of which the transition from the second to the third stages of medical education, essentially from pre-clinical to clinical education, would be restricted by tougher and more formal examinations.

Law and economics students were persuaded onto the streets in Paris last week by a similar provision in the proposed law on university reforms that would restrict students' transition from the first (two-year) cycle of university education to the second. Article 13 of the draft law, which has not yet been debated by the National Assembly, would have the numbers of students making this transition restricted by the capacity of university departments to educate them as well as by the opportunities for professional employment after graduation.

The promise that last year's decree will not begin to apply until 1984 means that medical students will not be affected until the end of this decade, by which time there will no doubt be room for further negotiation.

Whether the government's concession on that front will also mollify the law and economics students who seem to have taken the lead in last week's demonstration is not yet clear, but the debate in the assembly will provide further opportunities for compromise without loss of face by the government.

Another disputed issue is the government's plan to encourage entry to universities without a formal school-leaving qualification (*baccalauréat*). Although students qualifying by means of work experience now amount to 12 per cent of the total, many hold that the government's plans mean a devaluation of *le bac*. □

Ohio radiotelescope

SIR — Your news item on the Ohio Wesleyan University–Ohio State University radiotelescope (*Nature* 10 February, p.455) was generally factual but made some inaccurate inferences.

First, you suggested that Ohio Wesleyan made an impulsive decision to sell the land "out from under" our neighbours at Ohio State. This is not true. Ohio State knew of our intention to look for potential buyers as early as 15 May 1981, when I sent a letter to former Ohio State president Harold Enarson. In addition, former OSU provost Ann Reynolds told me in January 1982 that her university was planning to cease operation of the telescope by 1984. Based on those and subsequent communications, the Ohio Wesleyan Board of Trustees directed me to proceed with the sale.

Second, you seemed to imply that the sale demonstrates a disregard on our part for scientific inquiry. Again, this is not true. Although Ohio Wesleyan is an undergraduate institution, our faculty is actively involved in research. In the sciences, for example, nearly half of our faculty is funded by outside sources including the National Science Foundation, National Institutes of Health and industrial and private foundations. A member of our science faculty is currently in West Germany on a Fulbright scholarship. Furthermore, several of our faculty hold editorial and elected offices within their respective professional societies.

We believe that research is vital not only for the personal development and professional growth of our faculty, but that it also complements the educational experiences of our students. Evidence of the success of our commitment emerged in the fall when Ohio Wesleyan chemistry students captured the top three — and six of the top eleven — places in a national analytical chemistry competition in Pittsburgh. Biology students from Ohio Wesleyan University have presented research reports at national meetings and published their results in established journals.

The decision to sell the land on which the radiotelescope rests was based upon what we believed to be a mutual decision by ourselves and Ohio State. The sale was conducted following careful deliberation and should not be considered evidence that we are retreating from our traditional commitment to the sciences and specifically to scientific research. THOMAS E. WENZLAU

Ohio Wesleyan University,
Delaware, Ohio, USA

Millimetre wave telescope

IN Sir Bernard Lovell's letter (*Nature* 21 April, p.650), Professor Smith's estimate in 1970 of the cost of constructing the 30-m millimetre wave telescope was misprinted. It should read "greater than £1 million", not "greater than £11 million". □

Extinction by comet or asteroid

SIR — In expressing his approval of Peter Smith's comments on rigid anti-catastrophism in geological thinking¹, Ken Hsü² takes him to task nevertheless for attributing to Alvarez *et al.*³ the idea that the Cretaceous was terminated by a *cometary* impact. Professor Hsü can rightly claim priority in this respect and with good reason postulate less catastrophic (10^4 yr) rather than instant extinction (10^0 yr) (compare ref. 4).

The real issue at stake is rather more subtle than a straight distinction between Hsü's *comet* and Alvarez *et al.*'s *asteroid*. Thus, the proposal by Hsü⁵, following Urey⁶, that extinctions are caused directly by comets may be excluded on general grounds because asteroidal impacts are both an order of magnitude more common and consistent as to rate with the incidence of major extinctions (five or six 10-km missiles during the Phanerozoic). Alvarez *et al.*, knowing this, suggested a *meteoritic* missile, also believing the asteroids in question (Apollos) derived from the asteroid belt. Wetherill⁷, however, had demonstrated that they are unlikely to be derived this way, so the cometary origin of Apollos proposed by Opik⁸ is to be preferred. On this hypothesis, Apollos are *cometary asteroids*, being comets deprived of their volatiles but not collisionally processed like meteorites. The Cretaceous period was thus most probably terminated by a cometary asteroid, as was indicated in our 1979 paper⁹ (compare ref. 10).

In this paper elsewhere^{11,12}, it has also been pointed out that no catastrophic theory of extinctions can be maintained if the "galactic" *episodicity* of geological phenomena is not predicted. We have shown that this happens in a natural way if the Oort cometary cloud feeding Apollos is actually dissipated and replenished each time the Sun passes through the galactic spiral arms.

Professor Hsü has clearly raised an important question but it is unlikely that evidence in the ground will readily distinguish between a comet or a cometary asteroid. The fundamental problem for theories of terrestrial catastrophism is therefore an astronomical one, whether spiral arms contains lots of comets.

S.V.M. CLUBE
W.M. NAPIER

Royal Observatory,
Edinburgh, UK

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Lake Mead moved

SIR — In regard to the *News & Views* article by R.D. Adams, "Incident at the Aswan Dam" (*Nature* 6 January, p.14), I would like to point out that Lake Mead, although on the Colorado River, is actually located in Nevada.

This of course presupposes that seismic activity did not translocate the lake from Colorado to Nevada.

DAVID R. WOODARD
Eisenhower Medical Center,
Rancho Mirage, California, USA

Precocious Newton

SIR — It must be assumed that Descartes reacted to Newton's "good luck" (*Nature* 14 April, p.557) by turning in his grave. For when Descartes died, Newton was a child of seven.

BASIL GREENSLADE
Bath, UK

Animal rights

SIR — Speciesism is an ugly word and speciousness is not much better. Your notice of Professor Peter Singer's arguments for more humane treatment of laboratory animals (*Nature* 24 March, p.287) makes sad reading, combining self-righteous superiority with the squalid device of guilt by association. You are sure that reviving of human volunteers as test organisms will not occur because "governments, societies and people would not allow such things to happen", as if counting the same people three times makes it true.

You suggest that Singer's "implied assertion that there is no sharp line to be drawn between people and other species of animals overlooks several simple truths". First among them you cite the example that populations of domestic animals are controlled "both as to number and to kind by people, not by the species concerned", as if that weakens, rather than helping to confirm, Singer's simple truth. Affluent and sophisticated Western societies can surely afford to look more carefully and more generously than our forebears at the many ethical and practical problems associated with human exploitation of animals, whether as pets, prey, the subjects of experiments or convenient devices for product quality control.

Please do not lend your weight to those already strong forces that press to close the watertight doors of self-interest as soon as a single voice obtains a wide public hearing on behalf of the great majority of the world's animals that manages its affairs without governments or societies and finds it prudent to treat people with a suspicion which your comments suggest is well merited.

HUGH BOYD
Ottawa, Ontario, Canada

On attributing consciousness to animals

Before we can attribute consciousness to animals we must first decide on our definition of the word.

John H. Crook*

WHEN we speak of consciousness in animals do we yet know what we mean? By "we" I mean "us biologists" and in particular those eminent practitioners who have recently re-opened the study of consciousness in academic psychobiology. In his seminal book *The Question of Animal Awareness*¹, Donald Griffin proposes that consciousness in animals can now be studied through modern research in communication, social behaviour and neurophysiology. Marian Dawkins in her study² of animal suffering shows how a study of animal preferences in choice situations provides an understanding of animal discomfort. Yet any *direct* knowledge of how an animal experiences its life is impossible. Some important philosophical problems are being deliberately set aside; almost as if we busy biologists can get on with the job on a basis of "Consciousness? — well you know what we mean!" and that behavioural indications of awareness can stand as evidence of animal experience. Can they?

Two recent conferences held to explore this problem continue to reveal an underlying dissatisfaction — almost an irritation — with an apparently intractable subject matter. At the Universities Federation of Animal Welfare (UFAW) workshop on "Self awareness in domesticated animals" held at Keble College, Oxford, in July 1980³ a definition of self awareness "seemed to be accepted as a working model". It stated that self-awareness is the animal's ability to abstract and to form a conceptual framework of its environment so that it can perceive itself and its actions in relation to the environment. However, all delegates agreed that no single experiment or set of experiments could conclusively prove the existence of self awareness in animals below the great apes. . . "suggestions were made which might allow the probability of its existence in certain species to be assessed". The report concludes "the subject remains a fascinating challenge in its own right . . . and is probably best tackled by systematically investigating . . . many areas". But can any investigations actually reveal how an animal experiences itself?

Likewise at the Dahlem Workshop on "Animal Mind — Human Mind" held in Berlin in March 1981⁴, Donald Griffin as chairman urged participants to devise experiments that would not only examine animal cognition more thoroughly but also provide evidence regarding animal ex-

perience. Do animals "ever think about images or representations of the outside world, examine them and make choices as to how their own behaviour might change the situation? Or are all non-human animals invariably in a state comparable with that of a human sleep-walker, responding mechanically, even though adaptively?" A bold question, for how could one ever *know*? At both conferences several members clearly continued to doubt whether this was a meaningful question. Furthermore the participants' usage of words concerned with subjective experience, emotion and feeling was often difficult to interpret in systematic ways. Perhaps the philosophical issues, so easily bypassed by empiricists, need closer attention after all. If we are to study animal awareness we must first of all agree in what respects it is meaningful to assert any knowledge of an animal's experience.

Experience and performance

In his manifesto for a modern psychology John Watson argued⁵ in 1913 that all words referring to unobservable subjective states, whether in animals or humans, should be eliminated from psychological discussion. The basis of the renewed science was to be the analysis of publicly verifiable behavioural events. Overnight, "consciousness" became irrelevant and to the hardest thinkers the term came to lack any meaning at all.

While the study of learning through reinforcement became a system of closed ideas, research on animal exploration gradually led to a conceptual revolution. The work of Tolman⁶ in particular showed that in exploring a maze a rat seems to make use of "maps" or conceptualizations and that comparisons between new experience and old knowledge was the basis of adaptation to novelty in space. Furthermore, exploration was a reinforcing activity in its own right, suggesting that the construction of conceptual "representations" was a major aspect of adaptive behaviour at least in mammals. Such work led to a gradual reappraisal of modes of explanation in animal psychology so that occurrences of cognitive processes and the possibility of mental states of subjective awareness could again be discussed by those deemed respectable in orthodox science. The inner world of experience anchored in the perceptual capacities of an organism first examined by Jakob von Uexküll⁷ thus became again a subject for investigation, along with the study of behavioural performance. But can

one ever infer the nature of *experience* from studies of *performance*?

We know that a cruise missile can travel over complex terrain performing elaborate "cognitive" processing, informational comparison and decision taking every bit as complex as that of a migrating insect or bird. A chess playing computer can perform cognitive operations at least as effectively as average human chess players. Yet most of us are not yet inclined to speak of consciousness in either the missile or the computer. Why, then, are we inclined to speak of it in animals, even perhaps in relatively lowly forms? Why not confer consciousness on certain computers?

At this point it is useful to take a closer look at how we use words referring to experience and performance. Experience words include consciousness, awareness, feeling and derivations of these such as happiness, sadness, suffering, joy. Performance words include response, reaction, movement, gesture, posture, speech, language. All experience words are in principle knowable only to the subject whereas all performance words refer to an object observed by a subject. Words in one category are not "translatable" into those of another; they do not carry the same implication. Thus, a "response" cannot necessarily imply an awareness. Awareness is present only when there is a personally known subjective contingency between a wasp sting (say) and the pain to oneself. When my companion winces as the wasp stings I may, on a basis of repeated experienced contingencies of this sort with myself as subject, think that he is feeling pain but I can never *know* whether I am right even if he confirms my suggestion. All experience words ultimately refer to personal events of which there is one subject only — myself. Every use of such a word with respect to another is an *attribution*.

By contrast, performance words, providing observers are agreed as to their referents, can be publicly confirmed. Based in sensation they allow empiricist enquiry, agreement or conflict between observers, hypothesis formation and scientific study commonly with a reductionist metaphysics. I *knew* you winced and it is publicly *confirmed*. Measurements and statistics become possible and the logical derivatives of scientific theory, hypothetical constructs and intervening variables, become woven into the logic of explanation. Watson's rejection of experience words was rooted in his empiricist stance.

Yet, in our daily interaction with fellow humans, we do not usually experience problems with attributing feeling states to them. It is an unquestioned habit. But what sort of meaning is it that attaches to propositions referring to experiences of *others* when experience statements only have meaning for *me* in that they are about my personal subjective condition?

Vesey⁸ provides a valuable synopsis of Wittgenstein's thought on this issue. It is not that absolute criteria for the meaning

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of particular "experience" statements can be established but rather that criteria do emerge from the common usage of language wherever experience words are in use. That we utilize criteria is certain, but what they may be or whether our assumptions of their accuracy are correct has always to be found out.

What, then, is the common usage of language where a relationship between experience and performance words is involved? Both categories arise from cognitions and it is only in the communications of these cognitions that the difficulty is disclosed. The propositions then in play are those concerned with relating feeling with observation. To reach a consensual understanding of such propositions greater negotiation is required in the case of words standing for experience than is the case for performance words.

Attributions of experiences to another may involve categorizations derived from knowledge of another's behaviour, situation or disposition and their similarities to mine: such attributions are based on inference from prior causes. But attributions may also predict what another may feel next or what he may do, and thus become forms of judgement. In communicating attributions, some or all of these cognitive activities may be in play and their accuracy may be confirmed by the actions or statements of others. The conventions of the time must also play a part in negotiations about the meaning of such propositions and become enshrined in consensual validation. At this level attributions emerge as the material of social representation and as elements in shared systems of belief⁹.

The attribution of consciousness to another is in fact a little discussed part of the wider problem of causal attribution. In psychological theory^{10,11} the causation of human action may be attributed broadly either to circumstances independent of an individual's motivation or to internal factors involving his ability, effort and intention which he may present as a reason for his action. The giving of reasons is taken to mean an awareness of self's position in a nexus of related events which include attributions of selfhood to others. The attribution of intentionality to a performer thus becomes virtually synonymous with attributing self-consciousness and the extent of intention present is one measure of the quality of consciousness¹².

Whenever someone performs an action there is the question: was it intended? The answer depends on an assessment of correspondence between the action and known personal dispositions of the subject¹³. Inferences based on close correspondence may be experienced as certainty by a perceiver, yet the extent of felt correspondence is clearly open to social bias. In the same way the attribution of self-consciousness to another involves an assessment of the state of an individual in relation to known "conscious" dis-

positions of people as they are generally accepted to be.

Sometimes the reasons given for an action by a performer do not appear sufficient to account for it, but may suggest hidden motives which could, if made explicit as in psychotherapy, be offered ultimately as real reasons. Such hidden motives remain "implicit"¹⁴. Similarly, in making attributions one may be inclined to suggest sometimes that the "real" intentionality and hence the working cognition of self remain implicit rather than explicit to the subject; he is not conscious as to the "real" reasons. As I shall argue below this viewpoint may be valuable in discussing the extent of self-awareness in animals.

In attributing conscious awareness to another person we thus require an experience of that whole individual. And if the quality of that consciousness is in question we examine our experience of the other to see if we can identify with his expressions in such a way that we *feel we know*. Ultimately, we appeal to all or almost all we know of the human condition when we make such attributions.

Cognition and attribution

At least three cognitive processes seem then to be involved in the attribution of consciousness to another: (1) Empathy. Certain emotional expressions of another call forth reciprocal states of feeling. (2) Analogical sympathy. Knowing that in (non-emotional) situation A, I experience X, I infer that when another is in situation A, he or she knows X also. (3) Concordance. Often two or more people agree about how they feel, based on their use of a family of feeling words used in the same way. One may thus identify with the feelings of a set of others.

The key feature of this process is the confirmation of our attributions through mutual checking. Words and feelings come together in a system of interpersonal meanings. But what is it exactly that is being attributed? The consciousness that is being perceived is evidently a "consciousness of". Consciousness has an object. Users of experience words normally imply an experience of something. Duval and Wickland¹⁵ talk of objective and subjective self-consciousness as two forms of awareness of one's self — as an object observable by others (body, behaviour, etc.) and as a subject (inner sensation). I suspect it is not meaningful to speak of "consciousness without an object". Such a term has been used to refer to states of consciousness uncovered, for example, in meditation; but I suspect that what is really present here is a "consciousness without intention".

Now, to be "conscious of" implies a process. When I speak of my consciousness I mean something like a picture or an interior sensation of which I both *have* awareness and of which I may say *I am* the awareness. If I also believe that my experience is contingent upon my central nervous system I will then also suppose that

there exists somewhere a mechanism that is performing the task of "doing" consciousness. But I can only suppose this if I concur with my fellow scientists in a belief about the body-mind relationship. If I am a sort of spiritualist I will believe something quite otherwise. Do biologists concur in this? I am going to assume (Popper and Eccles¹⁶ notwithstanding) that they do.

But, like Griffin's human sleep-walker or chess-playing computer, active organisms could be "on automatic" and produce all sorts of behaviour without knowing anything of it. For many activities, even complex ones, consciousness does not seem to be necessary. And indeed there are many things my own body does of which I am not aware — heart beats, for instance. So "being aware" only comes into play in certain contexts of my life. What are those contexts?

Humphrey¹⁷ thinks they are primarily social. Organisms, he argues, are most aware of those social acts and inner conditions which they need to experience in order to understand the correspondent communications they receive. The value of internal awareness lies in the insight such knowledge gives to the functioning of others. Advanced social animals need to communicate as it is adaptive for them to relate themselves to others within a community. I agree largely with this and conclude that awareness must involve a second order representation of selected sensations (stimulation, bodily conditions, memories, physiological conditions, thoughts) for interior survey at particular times. The human capacity for self-awareness has probably evolved as part of our social apparatus and elsewhere I have argued the case for a derivation of human self-consciousness from reciprocal altruism in primates¹⁸.

To attribute consciousness to another person is therefore to use certain criteria in communicating a belief in the presence in another of such a function. And to attribute forms of consciousness to animals is but an extension of this.

Evolution of awareness

We have no knowledge about what the mechanism for such representation may be, although several contributors to the Dahlem conference allude to ways of approaching this problem. To talk of consciousness is thus rather like talking about heredity before cell biologists had discovered the significance of the chromosomes.

Ethologists comparing animal cognitive abilities with those of humans have sometimes found it helpful to look first at human abilities and then observe the extent to which animals may resemble them^{19,20}. The most salient aspect of consciousness with which humans are concerned is "self-awareness".

In 1890 James²¹ distinguished clearly between an awareness of the self as "me" — a socially defined entity with properties

such as a face, body, clothes, a house and a bank balance, as well as intangibles such as feelings and intelligence — and the self as ‘I’, an interior awareness, almost a proprioception of oneself as being. Research focused at first mainly on the ‘me’, with Cooley²² and Mead²³ emphasizing that self-awareness is totally concerned with the opinions and evaluations of self by other people. These and later ‘symbolic interactionists’ saw self-awareness as originating in the early struggles to relate self to others in a social environment. Experiments show that individual persons have a strong tendency to adhere to the cultural expectations of their social milieu. Contemporary self-awareness research²⁴ takes a broader perspective based in experiments showing that anything or any condition in which a person is reminded of self becomes a possible source of self-awareness.

Ontogenetically, awareness of self as an agent appears to arise through the learning that responses of others that are reactions to one's own behaviour are contingent upon a performance that is ‘mine’²⁵. Among humans this knowledge probably only begins to play a critical role in determining action tendencies that become permanent features of a personality when conflict between self and care-giver produces an awareness of another's judgement of self's actions. From this point on the identity struggle to maintain a positive sense of self-esteem begins.

Sporadic research suggests that cognitive abilities approaching these may exist only in animals as mentally advanced as the great apes²⁶. Are we then to assume that if consciousness exists in animals other than these it is radically different from our own?

Duval and Wicklund¹⁵ also examine the ‘subjective self-awareness’ of humans that James called the ‘I’. This is an awareness of self in action (driving, etc.) where the inspection of self as an object of consciousness is absent. This is a task rather than a self-oriented mode of being. In this state highly elaborate decision-making and information monitoring processes may be carried out in which the consciousness of self as agent is an implicit rather than explicit awareness. The attribution of this form of awareness to numerous mammals (cats, elephants, etc.) seems entirely reasonable. But where now do we stop?

There are two aspects of evolution to be considered: adaptive radiation and the emergence of complexity. At any taxonomic level adaptive radiation into a variety of habitats may include the evolution of a variety of societal conditions²⁷⁻²⁹. Most ethologists would probably agree that animals with complex societies also evolve elaborate communicational behaviour which may well require consciousness for adequately adaptable performance. Humphrey¹⁷ states categorically that only animals which have the capacity to advertise their feelings in expressive performances are likely to have consciousness and this because it helps them to

understand the expressions of others.

It seems unlikely, however, that less social animals are necessarily less conscious. Their consciousness may differ not so much in its level as in the specifics of the environment which the species' mechanisms of selective attention present for conscious evaluation. The events and situations that bring about alert attention in dogs and in cats are manifestly different, yet we are likely to attribute high levels of consciousness to both. Representational modelling of group behaviour may be an important focus on inner attention for the sociable dog, while modelling of the local environment with less focus on social relations may be characteristic of the cat.

Social representation is after all but one aspect of environmental representation, and animals in complex societies are thus probably not the only creatures in need of a little ‘natural psychology’. Research on kestrels has revealed the extraordinary precision of their spatio-temporal mapping that enables them to patrol their home ranges with a foraging strategy neatly geared to the positioning and timing of prey availability³⁰. The complexity of such space-time contingencies and the need to monitor environmental change may well involve an attention involving awareness, perhaps resembling a viewing of a plan position indicator in a radar system. Such awareness may lack any reference to self as agent in an explicit form but there may well be an awareness of an implicit sort since the temporal and spatial positioning of self is vital to the calculus of effective foraging. We could perhaps envisage self-consciousness remaining ‘implicit’ in the more solitary foragers and emerging explicitly only in nature's advanced sociologists.

But is there also awareness where responsiveness alone appears to give evidence of what in ourselves we would term sensation? The question here concerns when in evolution it might pay an animal in terms of fitness to evolve that extra bit of neural apparatus that awareness would seem to require. A sequence of stimulus-response behaviour may create the impression of an animal's having sensations, yet it may be more economical in the currency of fitness for it to be quite automatic. Where, then, is the provision of an overseer required?

My guess is that overseeing a decision process becomes evolutionary sense in the context of certain dissonance situations when, prior to a decision being made, information of several different types has to be integrated. Dissonance arises when there is a mismatch between an ongoing expectancy based on prediction and an actual situation. Rapid calibration of behaviour in relation to circumstance is then required. A ‘pictorial’ representation of the entire data set with a superimposition of changing states would be valuable in detection and adjustment and provide multilateral rather than linear processing. Some of the required information would concern internal

states and their representation would take the form of feelings generated from the inputs of physiological monitors.

Such a concept means that constraints on awareness would vary adaptively between species. Attentional processes evolved through natural selection would select only certain categories of information for representational oversight. At any one time the content of an individual awareness would be governed by rules of salience which would provide a hierarchy of criteria determining priorities for attentional ‘screening’. I am arguing that awareness is the subjective *aspect* of such a representational process and that there is no ‘ghost in the machine’³¹ additional to this.

Consciousness in evolution

When may ‘we biologists’ reasonably attribute awareness to an animal species? Five levels of conscious awareness seem possible, only the last two approaching the criteria of the UFAW conference and Humphrey's usage of the term ‘consciousness’.

● **Proprioceptive body awareness:** Closed loop systems monitoring the interdependence of bodily processes involve a continuous attention to inputs from many sources — as in bodily positioning in aerobatic flight or in echolocational pursuit. An ‘inner feel’ for the bodily state may be an important aspect of attentional surveillance where mismatch monitoring is essential for rapid decision making³².

● **Awareness of situational contingency:** At times of intense activity an awareness of the relations between bodily positioning (as above) and environmental situations (danger, prey attack, etc.) may be concomitant with movement and provide an implicit reference to self's position.

● **Awareness of agency:** A continuous monitoring of body positions in time and space suggests an implicit recognition of body as agent in the tactical expression of a foraging strategy³⁰.

● **Awareness of social agency:** A continuous monitoring of the relations between self and other in complex mammalian societies is highly likely to occur in the context of collaborative, manipulative or cheating behaviours¹⁸. Again, such a process will tend to allow the insight that the bodily agent is the subject of proprioceptive and situational awareness.

● **Linguistic self consciousness:** Here the insight relating inner feel and agency in behaviour leads to clear symbolization of *self* as agent and the use of pronouns in language. As language develops, the use of metaphor in expressions of knowledge means that consciousness becomes primarily concerned with meanings and awareness constrained by words.

The extent to which these forms of awareness are present in a species will depend on its evolved capacities, the categories of information selected for inner-display, the hierarchy of criteria regulating priorities for attention and other contingencies such

as health, stress and attitude.

In what way can research deepen our understanding of this subject? Some recent studies suggest a way forward in which precise descriptions from field and enclosure studies reveal the interactions with which animals form and maintain relationships³³. Such research pinpoints the inferences that an animal must be making if it is to opt for behaviours that establish relations with social partners in ways that are strategically beneficial. Recent work suggests that macaques and baboons can assess certain aspects of the relationship between two *other* individuals. This is especially apparent in so-called triadic relations where the action of a subject depends upon some understanding of the relation between the other two³⁴⁻³⁸. Colvin²⁰ has studied in field conditions the extent to which a young male rhesus monkey would respect or interfere with a dyad of two male peers. The best hypothesis to explain the results assumed that the degree of restraint or interference depended on an assessment not only of the familiarity obtaining between a male and each of his peers but also that obtaining between the peers themselves.

As Colvin has argued, this implies the possibility that simple representations of self might be generated by these animals as a direct result of comparing objective representations of others' relations with indices of the individuals own relationships to them. The step to self conceptualization would necessarily require a conversion of such indices from a non-representational to a representational condition. Such a step would mark the transition from implicit to explicit self-awareness.

Comparisons of the behaviour of socially complex animals with that of less socially complex taxonomic relatives would suggest species and circumstances in which conceptualizations of self may be emerging. Experimental manipulations of captive groups may then become a productive form of investigation. Studies of laboratory chimpanzees already show how subtle an individual's appreciation of its relation to a trainer's proffered attitude can be³⁹ and reveal the high levels of intentionality possible in these animals^{40,41}. All these issues require much further study.

Ethics of animal exploitation

Will it ever be possible to move from a mere attribution of awareness to a more certain knowledge? This will only come about as we begin to probe the neurophysiological processes involved in awareness of different types and levels. I predict that, just as ideas about hereditary factors preceded the discovery of genes, so our tentative analyses of consciousness will give rise to discoveries in the neurophysiological domain. It is not in communicational studies alone that the answer will be found but in a much enhanced understanding of brain functions, especially in sleep and waking. If some gadgetry providing the capacity for

self-reported awareness in our own species is discovered, then homologues or analogues of such mechanisms in other creatures will become powerful criteria for attributing experiences of perhaps specifiable types to them (see Dahlem conference). In the meantime, we should beware of those hard-nosed empiricists who still favour a science in which only public data are to be discussed. They now inhibit progress in cognitive research.

There is, however, a danger in the imprecise use of experience words. Computer specialists, whose jargon sometimes seems to have become an entire way of seeing the world, are often tempted to absorb common language terms and assimilate their meanings as inappropriate metaphors. Inventors of chess-playing machines or robots may not only claim that their creations think (meaning that they manipulate information to make decisions) but that such performance also merits the attribution of consciousness to them. A narrowing of the concept of consciousness in this way is a real possibility and one that should be resisted for the sake of the whole language of the human heart and poetry.

Several arguments against this impropitious use of words are available. First, all organisms share a self-referential cybernetic physiological system evolved to ensure self-maintenance at least until reproduction, and autonomous self-reference in cognition is a direct extension of this. Computers, by contrast, refer all their activities to their creator — controller and are not (yet) designed for independence of action⁴². They are in effect simply extensions of their creator's cognition. Second, while natural languages are open and become meaningful through negotiation and consensual validation between interdependent persons, computers use closed language of fixed meanings. There are thus categorical differences of a major order between protoplasmic and hard-wired cognitive processes and the attribution of experience words referring to living beings to machines is at present entirely inappropriate.

Finally, if forms of subjectivity are indeed aspects of the attentional processes found in animals, then in varying degrees animals resemble ourselves and our feelings of kinship to at least some of them are by no means misplaced. Certain logical consequences follow. If we wish to limit the suffering of living beings this wish must also refer to animals other than human ones. The process whereby we persuade ourselves to exploit animals painfully resembles that by which we depersonalize human opponents in order to kill them. Traditions of humanism rest ultimately upon compassion. A recall to conscience of those values that guide our behaviour to one another may be the wisest course in legislating our control over the lives of animals. Equivocation in either case devalues our quality of being and stems from the same willingness to create distance

whenever to hurt is profitable. If we perceive other forms of life as being to varying degrees in the same boat as ourselves, we have to decide on the width of the term "we".

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Chaos, a problem for experiment

The study of chaotic behaviour has thrown up a new vocabulary, and some intriguing concepts. But recognizing chaos experimentally may be more difficult than has been supposed.

If thinking the unthinkable has become respectable, surely defining the undefinable is a goal at which to strive? That, no doubt, is the spirit in which Li and Yorke embarked in 1975 on their already classical definition of chaos (*Am. Math. Monthly* **82**, 985; 1975). The concept of chaos has since then emerged essentially distinct from the most familiar physical manifestation of disorderliness, randomness as made manifest in brownian motion. The interest, and importance, of what is now called chaos is that it arises as a consequence of strictly deterministic processes. Everything is calculable, and thus in principle predictable, but our ways of comprehending chaos are poorly developed. So by what conceit can Gribogi, Ottand and Yorke now claim (*Phys. Rev. Lett.* **50**, 935; 1983) to have identified a novel kind of chaos?

Nobody denies the underlying practical importance of chaos in dynamical systems. In calculations of the evolution of meteorological patterns in the Earth's atmosphere, for example, experience in the past few years has shown that prediction and reality diverge after 10 days or so, not so much because the underlying dynamical system is incalculable but because the pattern of evolution is extremely sensitive to the initial conditions, which are even now only poorly known. Chaos is also common in biology. Students of population dynamics, for example, have to their surprise now recognized that when the sizes of living populations fluctuate widely, even wildly, from one season to the next, the underlying explanation may again be some kind of chaotic behaviour.

Why is all this comprehended only with such difficulty? Part of the problem is that we are trapped by what Thomas S. Kuhn would no doubt call the paradigm of newtonian calculus.

Take an inclined surface (not necessarily a plane) bounded by two edges at the "top" and "bottom" of the incline, and conduct the simple experiment in which billiard balls are allowed to roll from various points A on the upper edge. The objective is to establish the relationship between the set of points A along the upper edge and the set of points B along the lower edge at which the billiard balls arrive. Ordinarily, if the inclined surface is reasonably smooth, to each A there will be a distinct B , so that the experiment will establish a mapping of the set of points A onto the set B , which is tantamount to saying that there is a functional relationship between them. Formally, $B = f(A)$, and it is possible to calculate f from the known

shape of the surface and the laws of motion.

Our common expectation is that if a billiard ball starts not from A but from the nearby point $A + \delta A$, it will finish at $B + \delta B$, near in some sense to B . Indeed, it is also natural to expect that A and δB will be related to each other in the sense that the ratio $\delta A / \delta B$ will be finite and that as δA becomes smaller (or tends to zero), so too will δB and in such a way that the ratio $\delta B / \delta A$ approaches some well defined limit, which is the differential coefficient of the function f .

This, the common expectation, should however be amply falsified by another equally common experience — that of the old-fashioned pinball machine in which an inclined plane is studded with an array of metal obstacles. The mapping of A onto B is then by no means orderly. Patches of the upper edge may be mapped onto the lower edge in such a way that they are jumbled up and may even overlap (so that it is no longer possible unambiguously to infer A from B). It may even be that δB is large even when δA is small, so that the function $f(A)$ will not be everywhere differentiable.

In the past eight years, much has been learned about the behaviour of chaotic systems by the study of simple mechanical and mathematical models. At the same time, there has emerged a novel and intriguing vocabulary. Bifurcation is widely used, not so much for the effect of a pinball pin on the motion of a rolling sphere down an inclined plane but for the common observation that changing the parameters specifying a potentially chaotic system will usually force it into one of two alternative conditions depending on the initial conditions. "Attractor" is another old word used in a new guise to refer to those states that frequently crop up even in a chaotic system. And then there are "strange attractor" states of the system, determined by certain sets of initial conditions, in which chaos supervenes. Whimsically, the sets of initial conditions are called "basins".

What Yorke and his associates have now done is to construct a series of mathematical models of a chaotic system which has unexpected properties. The simplest case is that in which the positions of points in the complex plane are defined by the pair of recurrence relations $\theta_{n+1} = 2\theta_n \bmod 2\pi$ and $z_{n+1} = \lambda z_n + \cos \theta_n$ where λ is a number between 1 and 2. (The instruction $\bmod 2\pi$ simply implies that one chooses some initial value of the angle, doubles it and keeps only the part of it less than 2π .) The question, as with the observation of

spheres in a pinball machine, is to know how the set of all possible initial conditions is related to the eventual outcome of the recursion, and a few simple properties of the system are clear. If, for example, the recursion is started off with an angle that is zero, and if the initial value of z is say 1, the value of z_n will be λ^n , which diverges to infinity because λ is itself greater than 1. Indeed, almost all sets of initial conditions end up with z equal to plus or minus infinity, which are thus attractors for the system. The interesting question is to define the range of starting conditions from which some other outcome is possible.

Even with this simple model of chaos, some remarkable circumstances arise. First, the boundary of the basin is nowhere differentiable which, on the analogy of the pinball machine, implies that no $B + \delta B$ lies near B . Worse still, the length of the boundary, which is some kind of curve specifying z as a function of angle and which would then be expected to have a length of the order of 2π , turns out to have an infinite length — a property diagnostic of a set of points with non-integral or "fractal" dimension. Yorke and his colleagues promise to show evidence that the fractal dimension is, indeed, $2 - (\ln \lambda) / (\ln 2)$.

An elaboration of the simple recurrence relation leads to still more surprising consequences. With the same definition of the angles, but with z_{n+1} defined as $\alpha z_n + z_n^2 + \beta \cos \theta_n$, the boundaries of the basins again have fractal dimensions. Because of the z^2 term, every divergent point now diverges to plus infinity. For suitable choices of the parameters, there is also a set of starting conditions for which true chaotic behaviour supervenes. By a suitable choice of the parameters, however, it is possible to banish altogether truly chaotic behaviour but Yorke and his associates now report that even then, the semblance of it persists. They have been able to describe the behaviour of these systems only by numerical methods, but they have found that even when chaos is not strictly speaking possible, as many as 10^7 steps in the iteration of their formulae may be necessary before the influence of the attractor at infinity is able to take over.

The practical importance of this result is considerable, especially in the now absorbing question whether it is possible to distinguish experimentally between chaos and statistical randomness. For if quasi-chaos can persist for such long periods, telling the difference may in some circumstances be impractical. □

Electoral procedures

Preference and paradox

from Robert M. May

IT is easy to agree how a group of people should choose between alternatives in a 'truly democratic' way: one simply takes a vote. Things become surprisingly more complicated if there are three or more possibilities to choose from or to rank in order; the outcome of a voting process based on majority rule (with all people having equal voting power) can now depend on the details of the voting procedure. In particular, if one decides to choose a winner by successive pairwise comparisons, the outcome can depend on the order in which the comparisons are made.

Recorded discussion of these problems can be traced back to late-eighteenth-century France, a time and place where both mathematics and political theory were flowering. The two basic approaches towards choosing among more than two possibilities remain associated with the names of Frenchmen of that period¹. The Condorcet² approach is based on pairwise comparisons: one may, for example, make all binary comparisons and select the alternative with the greatest number of wins; or one may make a sequence of comparisons, discarding losers, until the winner emerges from the final binary choice. In the Borda³ approach each voter assigns points to each possibility, according to the position of the possibility in that voter's preference order; all points are then added up and the winner is the one with the greatest point total. As we shall now see, paradoxical situations can arise under both these schemes.

Suppose a committee of 11 people — or a community of 11 million people — is deliberating among three alternatives, labelled A, B and C. Suppose four of the committee members have the transitive preference order ABC; that is, they prefer A to B, B to C, and therefore (since their preferences are transitive or consistent) A to C. Suppose another five voters prefer the transitive ranking BCA, while the remaining two people have the preference order CAB. It will be seen that A beats B (6 votes to 5), B beats C (9 to 2) and C beats A (7 to 4). Thus although each individual voter has a transitive preference order, the group as a whole produces an intransitive ranking in which A beats B beats C beats A.

This phenomenon, sometimes called 'Condorcet's Paradox' or 'The Paradox of Voting', has many variations and has fascinated many people (including Charles Dodgson/Lewis Carroll) over the past two centuries. At the grandly philosophical level, the paradox undercuts any simple notion that democratic decisions can always be made unambiguously by majority vote. Following Arrow's writings on this aspect of the paradox⁴, it is often referred to in the

social sciences as 'Arrow's Impossibility Theorem'.

Equally, however, the paradox has implications for everyday committeeman-ship. Suppose the committee described above chose first to vote between A and B, and then to compare the winner with C. If everyone followed his actual preference at the first vote, A would beat B and finally would lose to C. Foreseeing this, a shrewd ABC preferer would do better to vote for B over A in the first division, with a view to B beating C in the final vote; the outcome B is better than C, which this voter would otherwise get. Even better for the ABC preferer, of course, would be a sequence in which B and C were first compared, with A then defeating B in the final vote — unless clever BCA voters 'cheated' in the first vote, voting for C over B, in which case C would finally triumph.

Many ramifications of all this, and particularly the implications for sophisticated voting, are elegantly set out in Black's book on *The Theory of Committees and Elections*¹. Riker⁵ and others^{6,7} have given examples where such tactical niceties have arisen in the US Congress and elsewhere. The interested reader will undoubtedly have met the phenomenon — knowingly or unknowingly — in university or other committees.

Can the paradox be avoided by the Borda approach? Insight into the answer can be gained by returning to our imaginary committee of 11. Suppose each individual awards two points to his first preference and one point to his second preference. Then the overall ranking is B (14 points), A (10 points), C (9 points). But had C not been involved, or if the lowest-ranking candidate is removed, then the order of the first two is reversed with A beating B (by 6 votes to 5).

More elaborate instances of this basic phenomenon can easily be constructed, and it is even possible to find examples in which the Borda point-total order of the first $N-1$ possibilities is exactly reversed when the last-place candidate is removed. To dramatize this, Fishburn⁸ presents an explicit example where a group of individuals with transitive preference orders arrive at the following point-total ranking for seven candidates:

Andrews:	President Elect
Brown:	2nd
Carter:	3rd
Davis:	4th
Evans:	5th
Foster:	6th
Gardner:	7th

But, writes Fishburn, "Before these results can be announced, the election committee is informed that Gardner has just died.

Thus, acting in accord with the election rules, Gardner's name is deleted from the ballots and new point totals are computed, giving the following results":

Foster:	President Elect
Evans:	2nd
Davis:	3rd
Carter:	4th
Brown:	5th
Andrews:	6th

These and other related phenomena have recently been reviewed by Fishburn⁹. My general impression of this body of work, which embraces both analytical and numerical analyses⁷⁻¹², is that the various paradoxes — particularly the more elaborate ones — are not likely to be too common in practice. But their possible occurrence must always be reckoned with.

Incidentally, although tactical aspects of voting under the Borda scheme do not seem to have received much attention, there is clearly much scope for manipulation, especially if the list is long.

My interest in these questions was rekindled recently by participation in a Princeton University committee charged with producing a shortlist of ranked candidates. If such a ranked list is assembled by pairwise comparisons, an interesting (and to my knowledge novel) variation of the Condorcet Paradox can emerge. Returning yet again to our fictitious committee of 11, suppose candidates A and B are first compared, whereupon A is ranked above B. The next step is to interleaf candidate C in this list. If comparisons are made descending from the top of the list, C will be compared with A and the resulting ranking will be CAB. On the other hand, if comparisons are made by ascending from the bottom of the list, C will be compared with B to yield the final order ABC.

At first glance, it might seem that this dilemma can be avoided by instructing the committee members to re-order their individual lists to conform to successive committee votes; that is, they must place A above B once the committee has voted on that binary comparison. It will be seen, however, that crucial ambiguities remain.

The pivotal voters in this instance are the five BCA people (the ABC people will consistently place C last, and the CAB people will likewise place C first); if these five obey instructions by putting A above B (to get

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ABC) then the final committee list will rank the candidates in that order, ABC; if the five obey instructions by putting B below A (to get CAB), the overall ranking will be CAB; and if the hapless five simply switch A and B on their lists (to get ACB), then the final result is ACB. At this point it may seem a good idea to give up the Condorcet approach, and opt for the aggregate point-score methods of Borda. But, as we have seen, these methods have their own faults, being among other things sensitive to the length of the list.

We are thus left with the conclusion that there is no foolproof way for a committee operating by majority rule to rank candidates on a list. Commonsense does,

however, suggest some steps that can be useful. For one thing, time may be well spent in trying to argue out some partial or tentative consensus, rather than moving too crisply to one or other kind of vote. For another, it may be wise to keep lists as short as is consistent with their essential purpose, to reduce the scope for subtle (or even unconscious) manoeuvring. Failing all this, one can at least be alert to the above intricacies of the committee jungle. From that point of view, I was probably foolish to write this article. □

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Geomagnetic field

New view on oscillations of magnetic shells

from Howard J. Singer

VARIATIONS in the Earth's magnetic field with periods ranging from tens of seconds to around 10 minutes are classified as long-period ultra-low-frequency (ULF) pulsations. In 1954, Dungey¹ suggested that these pulsations were standing hydromagnetic waves resonating on geomagnetic field lines, and numerous ground-based and spacecraft magnetometer observations have since supported and enriched the standing-wave model. Since 1977, a new technique², the Scandinavian twin auroral radar experiment (STARE), has made it possible to examine the electric-field signature of the longest-period ULF waves (Pc 5) in the ionosphere at the foot of the field line. Among the STARE observations is a class of waves whose period increases with latitude for individual events³. These observations are important for understanding the source of the pulsations and the nature of the coupling of energy between individual oscillating shells of the geomagnetic field.

An important argument for the standing-wave model is that wave periods are consistent with the time it takes Alfvén waves to travel along field lines between ionospheric boundaries where the waves are reflected. The dependence of wave period on the background magnetic field and charged-particle mass density along the field line in the magnetosphere suggests the period should vary systematically with geomagnetic latitude. Indeed, ground-based observations have shown that although many pulsation events are essentially monochromatic over a wide range of latitude, statistically the longer-period events occur or have maximum amplitudes at higher latitudes. There are also some events which are composed of several discrete frequencies where the low frequencies dominate at high latitudes⁴. These two different types of observation have important

implications for understanding the wave source. For example, in the first case the source is likely to be monochromatic and in the second case broad-band. It is therefore essential to know whether the ground-based observations are a reliable indicator of what is happening in the magnetosphere.

One difficulty in interpreting the ground-based observations is that the field observed on the ground is produced by currents in the ionosphere. Not only is wave energy lost in the ionosphere through Joule heating due to Pedersen currents, but the ionosphere acts as a spatial filter⁵ and severely attenuates short-scale signal variations of less than 120 km. The STARE system is not subject to the ionospheric screening and in addition makes virtually simultaneous multiposition measurements in both latitude and longitude, making it possible to examine the spatial structure of the wave.

The STARE system^{6,7} can be used to measure the ionospheric electric field over a large area (400 x 400 km) with high spatial (20 x 20 km) and temporal (20 s) resolution. It consists of a pair of coherent pulse doppler radars located near Malvik, Norway and Hankasalmi, Finland. The principle of operation is based on the radar signal reflecting from electrostatic waves excited by instabilities in the auroral zone E region. The doppler shift of the backscattered radar signal is a measure of the electron $E \times B$ drift velocity which makes possible the determination of the amplitude and direction of the electric field in the overlapping region of the two radar beams. One limitation of the STARE system is a requirement that the electric field exceed a threshold value (15–20 mV m⁻¹) to excite instabilities which reflect the radar pulse.

In a recent survey of STARE data³, 49 transient events, rarely visible for more

than five cycles, were found where the individual pulsation events showed an increase in period with latitude. In one of the displayed events, the period changes from about 416 s to 500 s over about 3° latitude at 1630 local time. These were toroidal eigenmode oscillations characterized by azimuthal oscillations of field lines in a shell around the Earth, although the longitudinal extent of the shell is not well established. At the time STARE observed these toroidal waves (magnetic east–west perturbation in space) a ground magnetometer at Tromsø observed primarily a north–south perturbation. This result was consistent with theory⁵ that predicts a 90° rotation of the magnetic field due to Hall currents in the intervening ionosphere. In addition, Poulter and Nielson³ have used the variation of the wave period with latitude to demonstrate a technique for determining the dependence of magnetospheric equatorial plasma density on radial distance in the vicinity of the STARE field lines.

The simultaneous occurrence of oscillating magnetic shells with different frequencies over a few degrees in latitude implies a broad-band or impulsive source for these waves. Such features have been observed before in space by radially moving spacecraft⁸, but only in special circumstances (for example, two closely spaced satellites) is it possible to separate spatial from temporal variations. The observation that field lines on adjacent magnetic shells can oscillate at different frequencies also suggests a weak coupling of energy between the field lines and should be of great interest to wave theorists and modellers.

The STARE observations have contributed to our understanding of ionospheric effects on magnetospheric ULF waves and will continue to do so. In the future, it should be possible to compare STARE measurements of pulsations with latitude-dependent frequencies with observations from a latitudinal chain of ground-based magnetometers. The comparison should explicitly determine the degree of screening by the ionosphere for this type of event. □

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Deep-Sea Drilling Project Leg 90

The South Pacific Cenozoic

from the Leg 90 staff

PALAEOCEANOGRAPHY, one of the youngest branches of earth sciences, was largely born of the Deep-Sea Drilling Project and continues to be nourished by it. The problems are of global scope so the appetite for new material is large. Leg 90 of R/V *Glomar Challenger* recently cored eight sites in a tropical to sub-Antarctic coring traverse and may help at least momentarily to moderate that hunger. On arrival in Wellington, New Zealand, the holds of *Glomar Challenger* contained more than 3,700 m of ocean sediment cores, the highest recovery of any leg. The cores were collected on relatively shallow-water pedestals conveniently aligned from north to south and draped with uncomplicated middle-to-late Cenozoic calcareous oozes and chalks (Fig. 1).

The quality of the cored sequence was generally good, as measured by the minimal sediment disturbance and the

stratigraphical continuity (85 per cent of drilled sequence) resulting from the use of the hydraulic piston corer (HPC), until it refused to penetrate further, followed by the extended core barrel (XCB), which was being used successfully for the first time. This core barrel extends several centimetres beyond the main rotary drill-pipe bit, ensuring a less disturbed drilling environment ahead of the main water jets of the drill bit. The longest HPC sequence, at Site 588, is a record penetration of 315 m, sampling a total of 17 Myr from the Quaternary to the late-early Miocene with excellent recovery.

Leg 90 sites were placed in every major surface-water mass between the tropics and the sub-Antarctic, first of all to obtain a palaeoceanographic history for each water mass and secondly to maximize our ability to correlate across the wide latitudes between equatorial and subpolar regions. We also recovered sections to form a vertical

traverse at mid latitudes from water depths of 1,299 m (Site 590), 2,131 m (Site 591) and 3,196 m (Site 206 of Leg 21).

The vast bulk of the sediment consists of calcareous oozes and chalk (usually >97 per cent carbonate), largely composed of calcareous nannofossils and foraminifera. The value of these sections largely lies with their potential for high-resolution oxygen- and carbon-isotope quantitative micropalaeontological and biostratigraphical investigations. Taken together these sections represent the most complete and coherent latitudinal traverse of Neogene sections from equator to subpoles yet collected from the oceans.

Although most of the samples collected must await further analysis, we learned much about surface- and bottom-water palaeoceanographic events, biostratigraphy, evolution, diagenesis and sedimentation.

One surprise is the high sedimentation rates of biogenic carbonates within some time intervals, as high as 131 m Myr^{-1} in the late-early Pliocene (4–3 Myr BP) interval of Site 591. We have been able to make good intercore comparisons of accumulation rates. The early Miocene (23–16 Myr) exhibits average and fairly constant rates of mass accumulation. Mass accumulation began to fluctuate at about 15 Myr up to 10 Myr (middle Miocene). A major peak (up to $12 \text{ g cm}^{-2} \text{ kyr}^{-1}$) is centred at 12 Myr. The late Miocene (10 Myr to 4 Myr) is marked by fairly constant and average rates of mass accumulation, suggesting a rather uniform depositional environment. At about 5 Myr, near the Miocene–Pliocene boundary, mass accumulation rates again increased, and between 4 and 3 Myr (late-early Pliocene) there is a remarkable widespread and brief peak of mass accumulation of up to $27 \text{ g cm}^{-2} \text{ kyr}^{-1}$. We do not believe that this results from an artefact of the time scale because the latter would need to be changed substantially to effect the pattern of increased rates.

In most other sites the Quaternary exhibits much slower sedimentation rates, perhaps because of increased winnowing associated with stimulated oceanic circulation during this period. We believe that, for the most part, the Neogene accumulation rates in these sites reflect actual changes in calcareous biogenic productivity. They are not due to dilution from non-carbonate sources, nor do they reflect dissolution. For several reasons, we have also dismissed the possibility of the observed fast rates being due to redeposition from winnowed

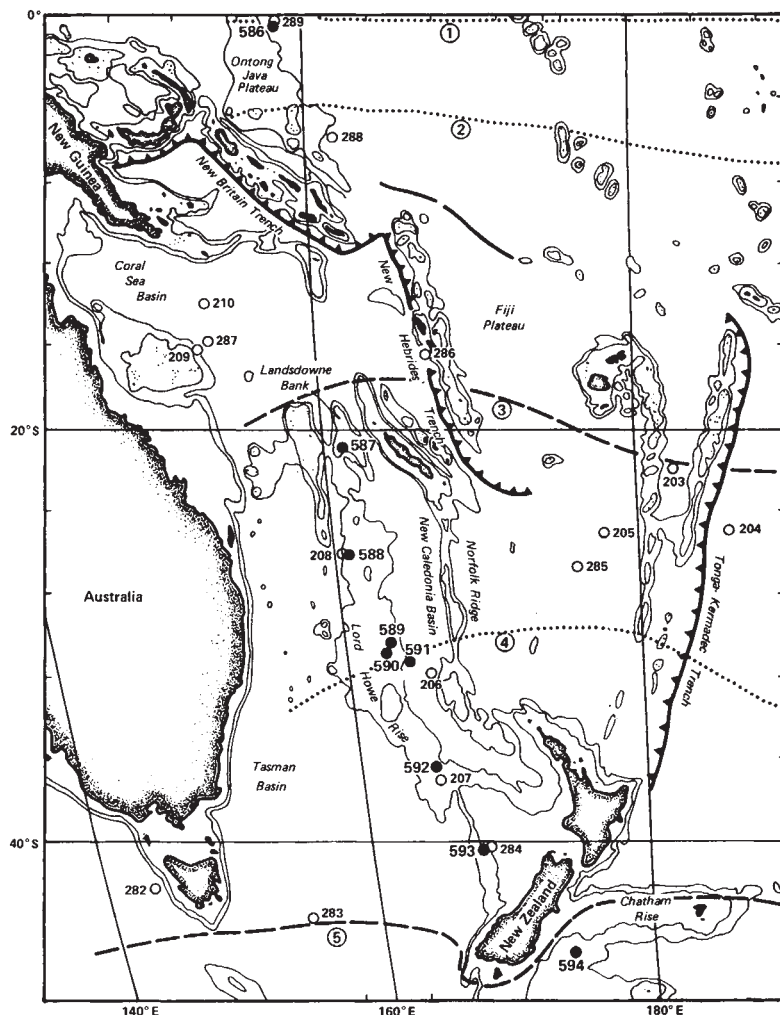


Fig. 1 Leg 90 (solid circles) and other (open circles) drill sites plotted on the generalized physiography of the South-west Pacific (1 and 2 km depth contours); approximate water mass boundaries are (1) Equatorial Divergence; (2) Southern Tropical Divergence; (3) Southern Tropical Convergence; (4) Subtropical Convergence.

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areas. The palaeoceanographic reason for such high productivity between 4 and 3 Myr is unclear because it occurred during a time widely believed to be marked by relatively warm uniform palaeoceanographic conditions before major Northern Hemisphere glaciation and ice sheet formation.

Most of the drilled sites contain easily distinguished layers of volcanic ash or altered volcanic ash. We were interested in determining the ages of the explosive volcanicity from the tephrochronology because this has been previously observed to be episodic throughout the circum-Pacific region. Site 591 has been examined in some detail. Figure 2 is a plot of the number of ash layers per million years for Site 591, ranging in age from the late-early Miocene (18 Myr) to the present day. Clearly there have been several episodes of intense volcanic explosivity: 18–14 Myr, latest-early Miocene to middle Miocene; 11–9 Myr, early-late Miocene; 5–4 Myr, latest Miocene to early Pliocene; 3–0 Myr, late Pliocene to present day.

The episodes of increased explosive volcanicity are separated by periods of relative quiescence which included some of the Pliocene and much of the late Miocene. The most important episodes are centred at 1.8–0 Myr (Quaternary), 10 Myr (late Miocene) and 15–17 Myr (early to middle Miocene). This explosive volcanic history is similar to trends previously compiled for the South-west Pacific as well as the circum-Pacific.

Our drilling was also concerned with the palaeotectonism of New Zealand. We

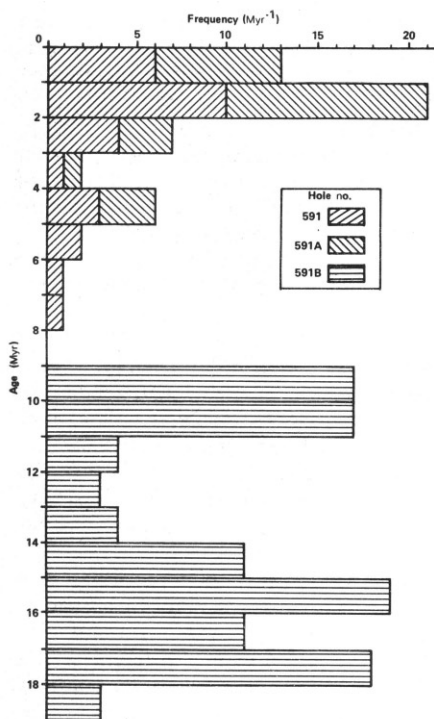


Fig. 2 Histogram showing the frequency per million years of ash layers and divitrified ash layers at Site 591 against core recovery. The thickness of each layer is more than 1 mm and typically several centimetres. Single very thin laminae less than 1 mm thick were omitted.

located Site 594 approximately 220 km to the east of South Island, New Zealand so as to monitor the changing influence of terrigenous and pelagic sediments through the Neogene. The first major influx of terrigenous sediments (hemipelagics) in the late Neogene was during the late Miocene at about 6 Myr. Until this date, late Neogene sediments lacked more than a minor terrigenous sediment component. Since the late Miocene, the terrigenous and pelagic in-

fluences have constantly oscillated.

We interpret the base of the hemipelagic sequence at 6 Myr to represent the beginning of uplift of the New Zealand Alps along the Alpine Fault. This orogenic phase, which led to the present topography of much of New Zealand, is the Kaikoura Orogeny. Our precise date for the beginning of this orogeny supports the more general observations on sequences in New Zealand. □

Molecular structure

Neuraminidase of influenza virus reveals a flower-like head

from Don C. Wiley

THE remarkable three-dimensional structure reported in this week's *Nature* by Colman, Laver and Varghese¹ for the influenza virus neuraminidase (NA) completes the atomic description of the surface glycoproteins of the virus. Now that X-ray crystallographic structures are available both for the NA and for the haemagglutinin (HA)² — the first two membrane glycoprotein structures to be determined — we may expect new insight into membrane surface activities: the glycosidase activity of the NA, and the receptor-binding and membrane-fusion activities of the HA. Furthermore, together with sequence studies³ and information about antigenic regions derived from monoclonal antibodies⁴, the structures allow a complete description of the antigenic structure of this animal virus that may provide the basis for an understanding of the antigenic variation which causes the familiar recurrent epidemics of respiratory disease in humans.

Before considering the biologically interesting features of these glycoproteins it is worth describing the NA structure because it is so unusual. It is a 4-fold symmetric oligomer of identical polypeptide chains with a box-shaped 'head' connected to the virus membrane by a long slender stalk. For crystallization the stalk was removed by pronase digestion, liberating the square head, comprising residues 74/78 to 469 and which retains full enzymatic and antigenic capabilities. The fold of the polypeptide chain is quite unique. Each monomer contains six β -sheets; each sheet contains four strands with the topology of a 'W'. Viewed from above the head, each monomer consists of six of the four-stranded sheets arrayed like the petals of a flower but twisted like the blades of a pinwheel. A new category of tertiary structure is born.

Although the three-dimensional structure of the stem is unknown, it may be even more unusual. Amino acids 1–6 are on the cytoplasmic side of the membrane followed by an uncharged and primarily hydrophobic peptide (7–35) which spans

the membrane, anchoring the protein to the lipid. This much of the structure is not unusual for membrane 'anchor peptides', although the N-terminal location is uncommon, the HA and most other viral glycoproteins having C-terminal 'anchor peptides'. It seems that the anchor also acts as a signal peptide during membrane translocation but is not removed by signal peptidase⁵. The slender stem, residues 36–73, however, is extraordinary both in containing 50 per cent of the oligosaccharides of the molecule (four sites) and in exhibiting absolutely no amino acid sequence homology among NAs from various virus strains — although the remainder of the molecule shows highly significant (> 50 per cent) homology⁶. All of this points to a relatively unfolded extended polypeptide and even the possibility of a flexible stem.

It may be noteworthy that both viral membrane glycoproteins, the NA and HA, exhibit novel structural features: the NA, a β -sheet pinwheel and a slender stalk; and the HA, a loop-like topology beginning and ending at the membrane, and a fibrous stem centred on an 80 Å long triple-stranded coiled-coil of α -helices.

The four carbohydrate chains in crystalline NA are distributed two on the top and two on the bottom of the box-shaped head. As in the Fc fragment of IgG⁷ and HA structures, one oligosaccharide is found at a subunit-subunit interface. Perhaps more striking is the observation that both on the NA and on the HA most of the oligosaccharides are attached on the lateral surfaces of the glycoprotein in positions where the glycoproteins might be expected to contact other proteins embedded in the same membrane.

The catalytic site of the NA has been located by difference Fourier analysis of crystals soaked in sialic acid. The site is surrounded by 14 conserved charged residues and contains three hydrophobic residues, Tyr, Trp and Leu, previously found in the sialic acid recognition sites of the wheat-germ agglutinin⁸ and influenza HA. The topology of folding in the NA is quite unrelated to that in the HA, however, so

suggestions that these two sialic-acid-binding proteins of the virus may have evolved from a common ancestor can safely be dismissed. The biological role of the NA activity is unclear. Without NA activity sialic acid remains on the viral glycoproteins where it binds the HA protein and leads to viral aggregation. Roles in transporting the virus through mucin and elution of progeny virus from infected cells have also been proposed.

Influenza virus is unusual because of the continual changes in the viral antigens that enable it to escape neutralization by antisera produced in earlier exposure, either through natural infection or through vaccination. Two modes of antigenic variation have been detected. Antigenic drift apparently results from point mutations in the virus genome which alter the surface glycoproteins in regions to which antibodies normally bind. Drift is associated with the familiar frequent recurrence of influenza epidemics. Much less frequently (1957, 1968, 1977), new virus strains appear in the human population by mechanisms (antigenic shift) which may involve genetic reassortment and/or transfer from an animal reservoir.

By examining the location of amino acid substitutions in a series of 'drifted' field strains and in three antigenic variants selected by growth in monoclonal antisera, Colman and colleagues have been able to propose the location of antigenic determinants on the NA. The antigenic sites are composed of loops connecting strands of β -sheet. They form a "nearly continuous surface" that "encircles the catalytic site" on the top of the NA. Although residues in the active site itself are conserved from strain to strain, the proximity of the variable loops to the site suggests that a significant part of the variable loops could interfere with any antibody contacting the active site cavity.

Although antibodies to the NA are not neutralizing, they modify the clinical symptoms of the disease. A similar analysis of antigenic regions has previously been done on the influenza HA to which neutralizing antibodies are directed². In that case, sequences from the major epidemics since 1968 and a large number of monoclonal antibody-selected antigenic variants when interpreted on the X-ray structure of the HA, indicate regions likely to bind antibodies. Since amino acid substitutions have occurred in all the regions between each major human influenza epidemic, alteration in each of those regions of the HA may be required for the production of a new epidemic strain.

Because so much of the glycoproteins' surfaces have been observed to vary in antigenically important ways, no simple strategy for disease control has emerged. Perhaps more important for novel antiviral strategies may be the nearly ubiquitous requirement of animal viruses for an entry pathway mediated by low pH into internal cellular vesicles⁹. If, as is the case in in-

fluenza HA, a protein conformational change induced by low pH is required for entry¹⁰, viruses may expose an Achilles heel only after uptake into cellular vesicles.

It is noteworthy that both viral glycoproteins have been solved by relatively novel crystallographic methods. The NA structure emerged only after data on two strains had been combined. One produced an uninterpretable map based on multiple isomorphous replacement phases, and in the other only a single isomorphous derivative and non-crystallographic 2-fold symmetry were available. An interpretable electron density map was produced only after the combination of all the data using real-space phase-refinement techniques^{11,12}. In the case of the HA, the crystals contained both a non-crystallographic 3-fold symmetry axis and an unusually high solvent content (78 per cent by weight), allowing the structure to be solved by only a single heavy-atom derivative and non-crystallographic

phase-averaging¹² incorporating solvent flattening. □

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Oncogenic intelligence

A new member of the *ras* family

from Peter Newmark

WITH the discovery that a human bladder carcinoma cell line (T24) contained a transforming gene closely related to the viral oncogene (v-Ha-*ras*) of the Harvey sarcoma virus of rodents, and that a transforming gene related to the oncogene (v-Ki-*ras*) of the Kirsten sarcoma virus was present in many human tumour cell lines as well as solid tumours, certain lines of research became inevitable.

One was to devise animal models in which the cellular *ras* genes were reproducibly activated. A start in that direction is reported on page 72 of this issue of *Nature*.

Another was to assign each of the human genes related to these viruses to particular chromosomes, a task that would have been completed by O'Brien *et al.* (*Nature* **302**, 839; 1983) who have now assigned four *ras* genes to four different human chromosomes — but for the discovery more recently of at least one more member of the *ras*-gene family.

The new member of the *ras*-gene family was identified during the study of human cancer cell lines whose transforming genes (detected by the NIH 3T3 cell assay) originally appeared to be unrelated to *ras* genes. Further studies have however revealed a relationship, if distant.

The strongest evidence of that so far has just been published from Michael Wigler's laboratory (*Proc. natn. Acad. Sci. U.S.A.* **80**, 2112; 1983) following up an earlier paper in the same journal (**80**, 383; 1983), and showing that the transforming gene of the neuroblastoma cell line SK-N-SH hybridizes to both v-Ha-*ras* and v-Ki-*ras*, though only weakly relative to the transforming genes of the T24 bladder carcinoma cell and various lung/colon carcinoma cell

lines respectively.

What appears to be the same new transforming gene, christened N-*ras*, has also been found in two human sarcoma cell lines by A. Hall, C.J. Marshall, N.K. Spurr and R. Weiss (*Nature*, in the press) who had previously been unable to detect hybridization between the gene and either viral *ras* gene (C.J. Marshall *et al. Nature* **299**, 171; 1982). Now, however, the weak hybridization to both has been revealed. Evidence that the sarcoma-cell-transforming gene is the same as that in SK-N-SH neuroblastoma cell came from the hybridization of a probe derived from the former with the latter but not with any other sequence in human DNA.

It has been assigned to a chromosome other than those identified by O'Brien *et al.* (*op. cit.*) as containing the other *ras* genes.

Both groups provide evidence that no major rearrangement has been involved in the conversion of the normal gene into a transforming gene. Hall *et al.* also provide evidence that the major transcript of N-*ras* is similar in size and quantity in their sarcoma cell and in normal fibroblasts, indicating that increased expression cannot account for transformation.

Most probably, therefore, normal N-*ras* is converted into a transforming gene by mutation, possibly by a point mutation equivalent to that which distinguishes the T24 transforming gene from its normal counterpart and which, according to a recent prediction (*Nature* **302**, 842; 1983), disrupts a potential GTP or other nucleotide-binding site on the protein encoded in the gene. □

Peter Newmark is Deputy Editor of *Nature*.

Protein evolution

Relationships of filaments

from Margaret Haugh and Brian Anderton

INTERMEDIATE or 10 nm filaments are new additions to the class of fibrous organelle that contains the microtubules and the microfilaments. The intermediate filament (IF) proteins are not highly conserved, unlike the microtubule protein, tubulin, and the microfilament protein, actin, and can be divided into five biochemically and immunologically distinct classes: cytokeratin, desmin, vimentin, glial filaments and neurofilaments^{1,2}. X-ray diffraction studies first led to the suggestion that IF proteins are structurally similar to the keratin-myosin-epidermin-fibrinogen (KMEF) group of α -proteins — both are thought to have structures involving extended coiled-coil α -helices³. Now, Klaus Weber and Norbert Geisler⁴ have found several remarkable similarities in structure between the IF proteins and wool keratin proteins despite their different functions.

The similarity appears to exist at two levels. First, in the gross organization of helical and non-helical domains, for which Weber and Geisler use the term 'motif'; and second, in a 70 per cent sequence homology detected in certain helical segments when they are aligned in the heptade manner. This is a manner of writing and comparing amino acid sequences of α -helices so that every seventh residue which falls on the same side of a cylindrical helix can be easily read and it makes it easy to depict the presence of hydrophobic residues in the appropriate sequential positions.

The motif for the IF proteins and the two wool keratins compared is simply that of a middle helical rod with non-helical head and tail pieces. The middle helical rod may be briefly disrupted by a non-helical segment, but if so the disruption is quite small, not as large as that suggested in an earlier model for IF proteins⁵.

Amino acid sequence data are available for three IF proteins: desmin, vimentin and the neurofilament 68,000-molecular weight protein (NF68). Sequence homology is apparent in fragments from the helical-rod section. By using a single tryptophan residue as a reference point for aligning the heptade arrangement, a remarkable sequence homology emerges. A 68-residue fragment from one of the wool keratins has homology of around 60 per cent with similar fragments from chick desmin, NF68 and vimentin. A smaller fragment (17 residues) from the amino terminal of desmin, both wool keratins and glial filament protein shows a homology level close to 70 per cent. The sequence of cDNA for human epidermal keratin has also been elucidated recently and when compared with the partial sequences for other IF proteins a 20–30 per cent homology emerges, suggesting a distant

relationship between the proteins⁶.

Weber and Geisler also point out, by way of contrast with the above similarities between these α -proteins, that the polypeptides have diverged to a considerable degree. This is seen both in the range of molecular weights found and also in the comparisons of the overall known sequences. One interesting aspect of this divergence is clearly related to known physical properties and, presumably, to function. The head and tail pieces of the wool keratins contain many cysteine residues (the middle helical region contains only a few) whereas in the intracellular IF proteins the head pieces appear to be rich in arginine residues. These features account for the known high level of disulphide

cross-links found in the keratin fibres and the weaker salt bridges and electrostatic links thought to stabilize the IFs. It seems plausible that the extracellular keratin would require the stronger covalent disulphide links for its stability whereas in the intracellular environment, which is reducing, disulphide bonding is rare.

It now seems legitimate to include the IF proteins in the α -protein group which could perhaps be collectively called KIFMEF proteins — any other offers from anagram enthusiasts to beat protein KEMIFFS? □

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Geology

Short ice age 230,000 years ago?

from J. T. Andrews

RECENT evidence for a brief but severe period of glaciation about 230,000 years ago is important for two reasons. First, according to Ruddiman and McIntyre, who have reported it¹, both the glaciation and the deglaciation were surprisingly fast; and second, it provides strong support for the Milankovitch hypothesis according to which Northern Hemisphere glaciation should coincide with insolation minima — periods when the Sun is at its furthest from the Earth.

Published summer insolation curves show that one of the strongest minima of the last 1,000,000 yr occurred 231,000 yr ago when the summer insolation at 80°N was -40 langley from the AD 1950 value. Insolation reached maximum values of about $+30$ langley on either side of the minimum with astronomically calculated dates of about 218,000 and 250,000 yr BP. This extremely sharp minimum occurs, however, within marine isotope stage 7 (refs 2 and 3) which, by-and-large, has been considered a nonglacial period.

The evidence that Ruddiman and McIntyre present is entirely from deep-sea records and they make no attempt to link their proposed event into existing glacial chronologies or stratigraphies. At some point the proposal of intense glaciation about 230,000 yr ago has to be positively associated with glacial events on land. It is important to distinguish between events recorded in the deep sea, that are the product of a globally integrated response to a climatic change, and more localized events. The recognition of a major change in the ^{18}O record at 230,000 yr BP tells us nothing

about where the glacial ice was stored.

This problem is both the beauty and the drawback of the deep-sea isotope record. It is essentially a black box that monitors and records several aspects of oceanographical change; it is not a simple measure of ice volume. If, for example, bottom-water cooling occurred in phase with the postulated interval of ice growth, then about 50 per cent of the isotopic signal that might be attributable to global glaciation could be explained by a 2°C cooling of the bottom water. Or, looking at it the other way, the actual amount of ice growth is largely unknown with ± 50 per cent.

Is there some independent index of ice volume that could falsify the suggestion of a rapid increase in global ice around 230,000 yr BP? The terrestrial glacial record is, unfortunately, a poor candidate for such a comparison. Dating methods that would give the required degree of accuracy and precision in this interval are largely lacking², nor does there seem to be any immediate prospect of accurately dating glacial and nonglacial sequences in the Hoxnian–Holsteinian interglacial interval.

Possible candidates for verification of the stage 7 glacial event are, however, offered by records of global sea-level fluctuations and high-latitude speleothem growth. Both depend on dating carbonates by the U-series method and involve precision errors of the order of 5–10 per cent. Harmon *et al.*³ reported U-series dates on speleothems from the Mackenzie Mountains of north-west Arctic Canada that indicate that the major peak in dates occurs at 200,000 yr BP, although there is a single

date at about 240,000 yr BP. Thus although the Arctic speleothem dates support the climatic marine implications of isotope stage 7 they are not ideal for discriminating between events on either side of stage 7b.

Variations in ocean level should be a first-order monitor of global ice volume, and although these records are more complex than had been thought⁶, they should be accurate enough for our purpose. Raised coral reef and submerged coastal speleothem records from subtropical and tropical sites⁷ do indicate two distinct high sea levels, at 200,000 and 230,000 yr BP, separated by an interval of low sea level. These data may thus serve to strengthen the arguments advanced by Ruddiman and McIntyre, although the actual date of the earlier sea-level high coincides with the postulated ice-growth events.

The remarkable speed of glaciation and deglaciation implied by the deep-sea record of stage 7b raises once again the question of how rapidly the world can proceed from an interglacial to a glacial mode. Ruddiman and McIntyre suggest that significant storage of continental glacier ice (possibly 30–60 per cent of the Late Wisconsin glacial 'maximum' at 18,000 yr BP) occurred in about 10,000 yr while the return to interglacial values occupied only 7,000 yr. Glaciological modelling of ice-sheet growth over North America for the last 125,000 yr BP has been attempted by Budd and Smith⁸, who use Milankovitch orbital variations combined with a relatively sophisticated glaciological model to investigate the extent and timing of ice-sheet growth for the Laurentide Ice Sheet. The process was found to be dominated by the 40,000 yr Milankovitch period — a finding partly supported by new evidence on the glacial history of Hudson Bay⁹ which suggests that complete glaciation and deglaciation of the geographical centre of the Laurentide Ice Sheet may indeed have occurred during the Last (Wisconsin) Glaciation.

But the evidence of Ruddiman and McIntyre requires processes to operate at twice that rate. How this might be accomplished climatologically and glaciologically is a moot point, but one that deserves serious consideration. Their paper should encourage detailed investigations into events during marine isotope stage 7 and the penultimate interglacial record on land. □

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Obituary

Charles Heidelberger, 1920–1983: research on cancer chemotherapy

from Peter Brookes

CHARLES HEIDELBERGER, Distinguished Professor of Biochemistry and Pathology and Director for Basic Research at the University of Southern California Cancer Center, Los Angeles, died on 18 January 1983, aged 62.

Charles Heidelberger studied chemistry at Harvard where he remained to work for his PhD in the laboratory of Professor Louis F. Fieser. He was no doubt exposed to that laboratory's enthusiasm for chemical carcinogenesis, although war-time emergencies had resulted in a temporary halt to this work. After a period in Professor Melvin Calvin's laboratory in Berkeley, where he became skilled in the synthesis of radioactively labelled compounds, Heidelberger joined in 1948 the recently established McArdle Laboratory in Madison, Wisconsin, where he remained for the following 28 years.

Charles Heidelberger devoted his entire working life to cancer research and made internationally recognized contributions in the areas of carcinogenesis and chemotherapy.

His early work involved the study of the metabolism of polycyclic hydrocarbons made possible by his synthesis of such compounds labelled with carbon-14. When he recognized that such studies were telling him more about detoxification than mechanisms of carcinogenesis he concentrated on the binding of carcinogens to cellular macromolecules and in particular the soluble proteins of mouse skin. He found for a series of hydrocarbons a good correlation between protein binding and carcinogenic activity; the only notable exception being for 1,2,3,4-dibenzanthracene. He realized that this apparent anomaly might allow him to identify the particular protein fraction most relevant to carcinogenesis and therefore initiated an extensive study of protein fractionation. This led to the identification of a class of skin proteins similar to the h-proteins reported by others to bind liver carcinogens. However despite considerable effort the significance of this protein binding remains undetermined.

While engaged in the above research Heidelberger became interested in the potential benefits to cancer patients of chemotherapy. He noted that uracil was used more efficiently by tumours than by normal cells. He therefore synthesized an analogue of uracil having fluorine in the 5 position, which would be expected to

block its conversion to thymine, required for DNA synthesis. He was able to show that 5-fluorouracil (5-FU) was active against a series of transplanted rodent tumours and subsequently its value as a clinically useful drug was confirmed. In later studies it was established that 5-FU was converted to the 2'-deoxyribonucleotide which was a potent inhibitor of thymidylate synthetase. Among many other fluorinated pyrimidines prepared in Heidelberger's laboratory, 5-trifluoromethylthymidine was shown to have powerful activity against several DNA viruses, including herpes simplex of the eye.

Recognizing the limitations of carcinogenicity studies *in vivo*, Heidelberger initiated work to develop a system of mammalian cell transformation *in vitro*. In his early experiments he used organ cultures of C3H mouse prostate, and when this failed to give tumours on reimplantation, he prepared cell cultures from the carcinogen-treated prostate. In this way permanent cell lines were established which did yield tumours when injected into C3H mice. Subsequent developments involved an aneuploid C3H cell line in a focus assay, and later a C3H line obtained by plating 0.5×10^6 cells per dish and transferring the cells every 10 days, following the procedure used by Aaronson and Todaro in establishing the BALB 3T3 line. By analogy this line was designated C3H 10T1/2, and shown to possess high post-confluence inhibition of growth and to have a very low transformation frequency. On treatment with carcinogen, readily recognized piled-up foci were produced, and this enabled the cell to be used in studies of tumour initiation and promotion.

Charles Heidelberger received many honours and distinctions. He was elected to the US National Academy of Science in 1978 and in September 1982 was the first awardee of the Athayde Cancer Prize. He was nominated as '1982 man of the year' by the American Cancer Society.

Heidelberger moved from Madison to Los Angeles in 1976 to help develop the new cancer center. He died without seeing the completion of this plan, but his contribution to this project in cancer research will long be remembered. □

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Chance, necessity and antibody gene dynamics

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Early in their development B lymphocytes commit themselves to the expression of only one immunoglobulin light-chain gene and only one heavy-chain gene out of the large numbers of available loci. Here I shall present the view that the initial selection of loci for expression is made by chance, whereas the exclusion of other loci from expression is a necessity imposed by the physiology of lymphocyte development.

HOW does one B lymphocyte make only one type of antibody?

The genes which encode the immunoglobulin heavy (H) and light (L) chains synthesized by B cells are created within those cells by genetic recombinations which, typically, fuse one of a hundred or so *V* gene segments with one of four of five *J* segments within a light-chain locus. In heavy-chain genes, a third element, *D*, is inserted between *V* and *J* (see Fig. 1).

The somatic recombination mechanism does not involve gene duplication, so that, for example, in a locus consisting of a *V* set, a *J* set and a single *C* gene, the *V-J-C* grouping necessary for expression can only be achieved once, removing all other *V* and *J* sets from the possibility of expression. Thus, a locus which contains enough information potentially to specify thousands of different *V-(D)-J* combinations is restricted to express only one such combination.

Consideration of other levels of genetic organization suggests that additional restrictions must exist. Each cell contains two parental alleles at each immunoglobulin locus, and, as was observed almost two decades ago, each individual cell expresses only one member of the allelic pair at each locus¹⁻³. Moreover, an animal may possess multiple non-allelic immunoglobulin loci; all mammals, for example, thus exhibit two types (isotypes) of light chain, λ and κ and again κ/λ (or isotypic) exclusion was observed to be a general rule for individual lymphocytes in the early 1960s (refs 4, 5).

Why have these additional restrictions on the expression of immunoglobulin genes evolved? Phenotypic expression of multiple light- or heavy-chain loci within a single lymphocyte would produce multiple H-L combinations with different antigen binding specificities. The consequent decrease in density of any one specific receptor at the lymphocyte surface could conceivably reduce the sensitivity of that B cell to stimulus by antigen to proliferate and secrete antibody in an immune response. Moreover, multimeric antibody secreted by such a cell, having subunits with different binding specificity within the same multimer, would be much less effective in agglutinating antigen. Below I will suggest another, quite different teleology.

The commitment made by developing lymphocytes to the exclusive synthesis of only one type of antibody may be considered in two parts: first, what is the basis by which particular genes and particular loci are selected for expression, and second, how are other loci excluded from expression?

Light-chain allelic and isotypic exclusion

Early suggested mechanisms of allelic exclusion included the notions that, by regulating chromosome disjunction and migration at mitosis, lymphocytes might achieve homozygosity and identity at immunoglobulin loci, or perhaps one allelic locus might be repressed in manner similar to X-chromosome inactivation (for example see ref. 6). However, analysis of a number of mouse plasmacytomas, each producing a single species of κ light chain, revealed many examples in which either transcrip-

tional activity or somatic recombination, or both, was evident and distinguishable in both κ alleles⁷⁻⁹. From these observations arose the idea that the somatic recombination process might be error-prone: not every rearrangement might form an effective *V-(D)-J* array. This admission suggested a minimal stochastic model of allelic exclusion: a rearrangement process, operating independently in both loci, might be so inefficient that the probability of achieving productive rearrangements within both alleles in one cell might be very small^{9,10}. Several examples of non-productive rearrangements have been described¹¹⁻¹⁵.

A survey of a group of 32 κ -producing plasmacytomas of the NZB mouse strain showed 17 examples in which the non-expressed κ allele was rearranged in some non-productive manner which is denoted as κ^+/κ^- , where (+) indicates productive, (-) non-productive rearrangement, and 15 examples in which the non-expressed locus was unrearranged which is denoted as $\kappa^+\kappa^0$, (0) indicating a non-rearranged locus^{9,16}. This ratio was imagined to be a function each of: the intrinsic rate of κ -gene rearrangement in B cells, the time elapsed during which rearrangement might occur and the relative probability of making a productive, as opposed to a non-productive rearrangement. Unfortunately, this simplest model became difficult to support when extended to cover κ/λ exclusion.

Analysis of a number of mouse λ -producing plasmacytomas and hybridomas indicated that the status of κ loci in λ producers is most frequently κ^-/κ^- (refs 17, 18). This is inconsistent with a purely stochastic mechanism operating to produce very few κ^+/κ^+ cells, and about equal numbers of κ^+/κ^0 and κ^+/κ^- cells: a rapid but highly erratic κ rearrangement mechanism would give more κ^+/κ^- cells, a slower but surer mechanism would give fewer κ^-/κ^- λ producers. It would appear that something extra is needed.

One way out of this difficulty is to adopt as a rule the restriction that acquisition of a productive rearrangement results in the cessation of all further rearrangement¹⁷⁻¹⁹, immediately imposing allelic exclusion as well as κ/λ exclusion. The simplest of such models is outlined in Fig. 2. A critical feature is that it demands that as *P* (the probability that any rearrangement will be productive) approaches zero, that is, the rearrangement process is extremely error-prone, the ratio 0+:--+ approaches a limiting value of 1. Note that the model shown in Fig. 2 does not include such extra, and quite realistic, complications as a (-) $\rightarrow$ (+) transition²⁰. One must also be aware that quite distinct influences will alter experimental data from that predicted by these formal models. For example, given the very rapid turnover of primary B cells^{21,22} it seems very likely that pre-lymphocytes also simply die, probably in large numbers, without undergoing either a productive rearrangement or a complete set of negative rearrangements at all immunoglobulin loci.

The best current estimate of the actual ratio 0+:--+ for κ genes in the κ -bearing B-cell population of the mouse spleen

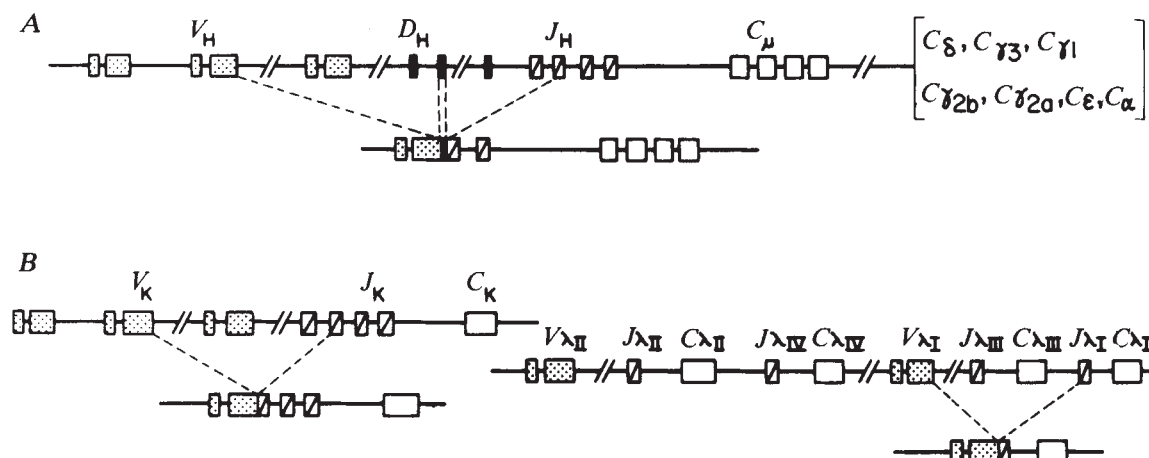


Fig. 1 A schematic diagram of immunoglobulin gene rearrangement. *A*, at mouse heavy (*H*) chain loci. *B*, at mouse light (κ and λ) chain loci. The three loci indicated are on three different chromosomes. There are probably a few hundred V_K elements in the κ locus, about the same number of V_H elements in the *H* locus with perhaps 1/10th that number of D_H gene segments. None of these numbers is known precisely, however. The mouse λ locus contains only two *V* genes and four *J*-*C* regions, of which one ($J_{\lambda4} - C_{\lambda4}$) may be inactive. The linear organization of these gene segments is not yet firmly established: that shown seems the most likely at the moment^{28,29,41}. Immunoglobulin gene expression requires precise fusion of *V*, *D* and *J* segments in the *H* locus, precise fusion of *V* and *J* in *L* loci, as shown. The exon shown upstream of each of the *V* segments encodes the signal sequence for a secreted protein. *J*-*C* fusion and removal of other introns occur during RNA processing. The λ -gene rearrangement shown, fusion of $V_{\lambda1}$ with $J_{\lambda1}$, is the one most frequently encountered of this locus⁴¹. With the exception of $C\delta$, stable expression of heavy-chain constant-region genes other than the C_μ requires additional DNA rearrangements which move the fused *V*-*D*-*J* segment close to one of the downstream C_H genes (see ref. 42 for a recent review).

is about 2 (refs 18, 23), implying $P = 0.5$ (non-productive to be as likely as productive rearrangement). However, the assay used is rather insensitive to decreasing P values below 0.5, and a 0+:-+ ratio as low as 1 could not be ruled out. As we have seen, this ratio is about 1 in a fairly large sample of NZB plasmacytomas, and actually significantly less than 1 in a further compilation of data about BALB/c plasmacytomas (taken from refs 8, 12, 13, 17, 24). A ratio below 1 indicates that some (κ^-) rearrangement must occur after the κ^+ rearrangement. In fact the occurrence of more than two arrangements of κ genes is quite frequent in the typically sub-tetraploid BALB/c plasmacytomas, and in some cases one can discern a probable precursor-product relationship between two of the loci^{8,24}. Admittedly, however, one must be cautious in ascribing all such rearrangements to the operation of the physiological *V*-(*D*)-*J* recombination mechanism, especially as tumour-specific chromosomal rearrangements may frequently occur close to immunoglobulin loci²⁵⁻²⁷. However, either the κ rearrangement system is extremely error-prone, or the shutdown of rearrangement on productive recombination is not absolute; or both. Presumably, in any rearrangements acquired after the initial productive (+) rearrangement, the prevalence of non-productive (-) rearrangements over (+) rearrangements necessary to maintain allelic exclusion, is once again dictated by purely stochastic considerations.

Can the operation of a mechanism like that in Fig. 2 then fully account both for allelic exclusion at light-chain loci and κ/λ exclusion? This issue is controversial. One view is that it can, and at some early phase of B-cell development, all κ and λ loci become available for rearrangement, which proceeds until a productive rearrangement is achieved within one locus, entailing a shutdown of rearrangement; κ and λ loci thus directly compete for expression¹⁸.

This cannot easily explain, however, why both in mouse and in man κ gene status is most frequently κ^-/κ^- in λ -producing cell lines, while in κ producers the λ genes are almost always unrearranged, λ^0/λ^0 (refs 16, 18, 19, 28). These data are taken to support an alternative 'developmental hierarchy' model of κ/λ exclusion. This model is also probabilistic, but κ genes are presumed to acquire recombinational competence earlier than λ genes. There are, however, wrinkles in the developmental hierarchy case: κ^0 loci have been observed in λ -producing lines, as has a λ^- locus in a κ producer¹⁸.

The concept that productive light-chain gene rearrangement leads to cessation of rearrangement is clearly a central component in the various models. Is this notion only a convenient arbitrary rule, or does it have some physiological validity? Little is known about the cellular physiology of B-cell precursors, but it is clear that, besides the ability to synthesize light chains, primary B cells do differ significantly from their precursors, pre-B cells. For example, pre-B cells which make μ heavy chain but no light chain, are actively cycling, while primary B cells are typically quiescent²⁹. If acquiring the capacity to make light chain is somehow responsible for triggering this transition, then a causal link between productive rearrangement and a battery of phenotypic changes, putatively including shutdown of rearrangement, is forged. Acquisition, through DNA rearrangement, of the ability of a cell to make immunoglobulin polypeptides might thus effect passage through a 'gate' from one phase of lymphocyte development to the next.

Assuming for the moment that this is true, one may ask: what features of light-chain gene expression are critically associated with the pre-B $\rightarrow$ B transition? The answer can be inferred from an analysis of plasmacytoma S 107 (refs 14, 15) in which the κ^- allele has undergone a *V*-*J* joining event that has deleted six nucleotides from the *J* coding block. This rearrangement allows correct transcription, processing and translation, yet secreted immunoglobulin contains only the light chain specified by the allelic κ^+ locus: the κ^- product, lacking two residues in *J*, seems to be unable to complex with heavy chain. Evidently the presence neither of κ RNA, nor of κ protein is sufficient to effect the pre-B $\rightarrow$ B cell transition; rather the formation of effective H-L complexes is necessary. One may imagine that the appearance of such molecules at the cell surface is needed to transmit some signal to trigger the transition.

Allelic exclusion at heavy-chain loci

To what extent does allelic exclusion at heavy-chain loci follow the rules suggested for light-chain loci? Although historically the light-chain loci were the first to be investigated, the heavy-chain genes are the first to become rearranged in ontogeny: the existence of a developmental hierarchy with a phase of μ heavy-chain gene rearrangement followed by a phase of light-chain gene rearrangement has been strongly established^{20,30-32}, and it seems possible that the (allelically exclusive) synthesis of μ chain is a predicate of a cell's gaining competence to

rearrange light-chain loci. Heavy-chain expression requires two joining events within one locus: one which fuses *V* with *D*, one *D* with *J*. Analysis of many B-cell tumours and of splenic B-cell populations reveals that rearrangement of both allelic *J_H* clusters is very frequent^{18,33-35}, so again, both alleles are available and presumably competitive for rearrangement. In some cases *J_H* rearrangement has been shown to be due to *D-J* fusion, in the absence of a *V_H* segment³⁶. Such *D-J* fusions resemble putative intermediates in the formation of a fully fused *V-D-J* locus. At present, it is not clear if *V-D* and *D-J* fusions can occur independently, or if *D-J* fusion must always precede *V* rearrangement, nor is it clear whether the statistics of μ -gene rearrangement will conform more closely to a purely stochastic model of allelic exclusion, or to one in which a successful (μ^+) rearrangement precludes further μ -gene rearrangement (ref. 32; as in Fig. 2).

Many questions remain unresolved but may not remain so much longer because of an exciting recent development.

Abelson virus, used *in vitro* to infect mouse cell populations rich in B-cell precursors, such as fetal liver or adult bone marrow, will frequently give rise to transformed cell lines capable of unlimited growth in culture. Phenotypic traits of many of these cell lines, notably the ability to synthesize intracellular μ chain in the absence of light chain, suggest that they may be transformed pre-B cells³⁷. The remarkable property of some of these cells is that they appear to be active in *V-(D)-J* recombination^{20,32}. The capacity to study immunoglobulin gene dynamics in cloned cell lines in culture will provide important insights, indeed it has already provided the strongest evidence for developmental phasing of heavy- and light-chain gene recombination³²: some fetal liver-derived lines rearrange *J_H* loci at a significant rate but are inactive in light-chain gene rearrangement, while in some bone-marrow derived lines the reverse is true. Furthermore, some of the light-chain rearrang-

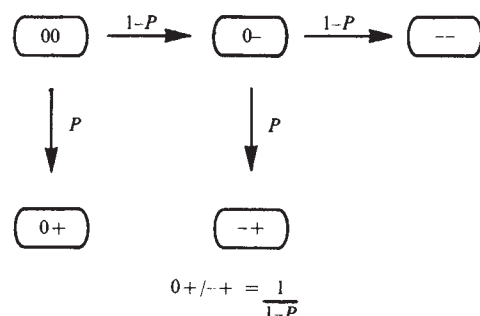


Fig. 2 Schematic diagram of a model of allelic exclusion at any immunoglobulin locus. 0 indicates a non-rearranged gene, +, a productively rearranged gene and -, an abortively rearranged gene. *P* is the probability that any rearrangement will be +, so that the ratio of the number of cells containing +0 genotype to that containing +-genotype is given by $P/(1-P)$.

ing lines have recently been shown to undergo rearrangement predominantly at κ loci and possibly not at all at λ loci. This has been taken to support the developmental hierarchy model for isotypic exclusion³⁸. Further analysis will allow estimation of the rate and fidelity of the rearrangement process at various loci and may resolve other vexing issues. Moreover, study of the enzymology of somatic recombination now appears feasible.

Immunoglobulin gene rearrangement in T cells

We have seen that the notion that the immunoglobulin gene rearrangement system is error-prone has been crucial in developing an appreciation of the rules which restrict immunoglobulin gene expression in individual B lymphocytes. Consider now the observation that *J_H* rearrangement is quite common in T cells³⁹. Although these rearrangements seem to have no physiological importance within T cells⁴⁰, might the apparatus responsible for their existence have a functional role in T-cell development?

The properties of those genes which encode the T-cell antigen receptor are controversial. It seems likely, however, that they have (at least) a common evolutionary ancestry with their B-cell counterparts, the immunoglobulin genes, and that they may also depend on some similar somatic recombination process for their clonally exclusive expression. The significance of *J_H* rearrangement in T cells may be seen in one of three ways: (1) μ genes and 'T-cell specificity genes' (*t* genes) depend on distinct mechanisms for their expression and *J_H* rearrangement in T cells is coincidental, (2) μ genes and *t* genes use a common recombination apparatus which, while rearranging *t* genes in committed T-cell precursors, occasionally accidentally rearranges a *J_H* gene and (3) μ genes and *t* genes use a common recombination apparatus and compete for mutually exclusive expression during a phase of lymphocyte development before divergence of B- and T-cell lineages.

In the third case, those rules developed to describe allelic and isotypic exclusion of immunoglobulin gene expression in B cells can be considered also to govern the acquisition of T-cell specificity. The decision to become a B or T cell is thus viewed as a stochastic process, essentially identical to the decision to become a κ or λ expressing B cell, or the decision to express the maternal or paternal κ allele; non-productive *J_H* rearrangement in T cells is then exactly analogous to non-productive κ rearrangement in λ -expressing B cells. Again, as before, the appearance of a μ or *t* gene product at the cell surface is presumed to trigger other phenotypic changes associated with B- or T-lymphocyte differentiation.

This model makes some interesting predictions: *t*⁻ rearrangements, for example should be evident in B cells, when appropriate probes become available, just as *J_H* rearrangements are seen in T cells. Furthermore, if initial synthesis of μ -chain or *t*-gene products determines commitment to the B- or T-cell

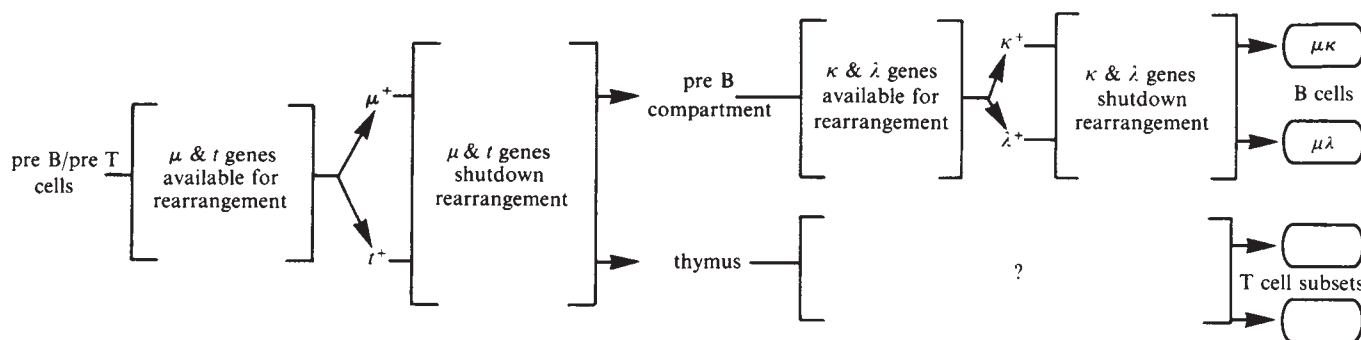


Fig. 3 A speculative diagram of lymphocyte development. Pre-B pre-T cells begin to rearrange μ genes and *t* genes. Successful rearrangement μ^+ or t^+ , leads to shutdown of rearrangement at both loci, and commitment to the B- or T-cell lineage. Committed pre-B or pre-T cells mature in appropriate compartments, where pre-B cells begin to rearrange light-chain loci. Again productive rearrangement leads to shutdown, entailing both allelic and κ/λ exclusion. Light-chain synthesis triggers the final stages of B-cell development, producing $\mu\kappa$ or $\mu\lambda$ primary B cells. A mysterious, but possibly analogous process generates various subclasses of primary T cells.

lineage, precursors committed exclusively to either pathway should not exist before this event.

Teleology of allelic exclusion

We have seen that the phenomenon of allelic exclusion allows a plasma cell to secrete more potent antibody than would otherwise be the case. Finally, I would like to consider how the error-proneness of somatic recombination may suggest another perspective on the teleology of allelic exclusion. The probability of any rearrangement being productive (P in Fig. 2) could conceivably be as low as 0.1. With such a low probability of productive rearrangement in all light- and heavy-chain loci, one might expect that only, perhaps, 5% of newly developed B cells would possess functional antibody molecules. This great wastage is avoided if the development of the B-cell phenotype is dependent on the ability to make antibody; those pre-B cells which abortively rearrange all immunoglobulin loci then can never become B cells. Exactly this concept was introduced above in an attempt to develop a model of allelic exclusion consistent with available data. Conceivably, allelic exclusion is a spin-off, convenient for its other advantages, of the economic necessity to channel lymphocyte development through gates

controlled by the ability to make μ chains, or heavy-light-chain complexes.

Figure 3 is a speculative synopsis of the role of gene rearrangement in lymphocyte development, emphasizing the idea that synthesis of antibody proteins may trigger cascades of phenotypic changes and allowing lymphocyte development to be presented in terms of elements of chance and necessity. Pure chance, translated into the stochastic process of gene rearrangement, governs, initially, whether μ or t genes will be rearranged, whether productively or non-productively, and later in B-cell development, which light-chain loci, allelic and non-allelic, will be rearranged, productively or non-productively. The selection of which gene segments will be fused within any locus, is also viewed as a stochastic process. However, if the choice of what rearrangement may occur is random, the consequences of that choice are ruled by strict necessity. Productive rearrangement commits a lymphocyte precursor to a definite pathway of development, fixing its fate as a B cell or T cell, a κ or λ -expressing B cell, a producer of the maternal or paternal allelic variant at any antibody locus.

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ARTICLES

Large-scale radio structures of superluminal sources

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Westerbork Synthesis Radio Telescope maps of faint extended emission associated with several superluminal radio sources are presented. In order to avoid exceptionally large sizes for the superluminal sources, their dominant, long-term axes must be at least 20–30° from the line of sight. This is a considerably larger angle than that demanded by the relativistic beaming interpretation of the superluminal motion in their compact cores, and implies that a wobble or precession of their ejection axes has taken place. Superluminality may thus be a temporary phenomenon.

It has become generally accepted that superluminal motion is observed in the nuclei of some radio sources. Components in the radio brightness distributions of NRAO 140, 3C120, 3C179, 3C273, 3C279, 3C345 and probably BL Lac appear to separate with speeds greater than that of light^{1–3}. Hybrid maps of 3C120, 3C273 and 3C345 at 5, 10.7 and 22 GHz (refs 4–6) show that the m arc s morphology of these objects can be generalized as an unresolved component (core) at one end of an elongated jet-like structure which has knots embedded in it. Maps at successive epochs reveal that it is the knots in the

jet and the core which move away from each other. Whether this relative motion is, in fact, due to the knots moving away from a stationary or quasistationary core is unclear in the absence of radioastrometric data, but it has widely been assumed that this is the case and that the structures are one-sided jets reminiscent of those seen on much larger scales.

Of the many theories devised to explain superluminal motion (see summaries in refs 1, 7, 8) those encompassing relativistic bulk motion of radiating electrons along or near to the line of sight to the observer have become the most popular because

on these models^{9,10} both the superluminal motion and the one-sided morphology have a natural explanation. Furthermore any true change in orientation of the approaching jet is magnified by a factor $\text{cosec } \theta$ (θ is the angle to the line of sight subtended by the jet) and this has been invoked¹¹ as the explanation of the large changes in position angle of the jets from m arc s to arc s scales.

Increasing interest is being shown in the larger scale radio structures associated with the superluminal sources because these reflect the long-term behaviour of the sources and may give some clue to the processes observed at m arc s scales. Previous observations have shown one-sided structure on arc s to 10 arc s scales in NRAO 140, 3C120, 3C273 and 3C345 (refs 12–14), and two-sided structure in 3C179 and 3C279 (refs 12, 13). We have made high dynamic range observations at 6 cm of five of the six sources with the Westerbork Synthesis Radio Telescope (WSRT) in redundancy mode¹⁵; 3C179 was observed in standard mode. We have discovered that, in addition to 3C179 and 3C279, NRAO 140, 3C120 and 3C345 also have components on both sides of the core. Observations by Zieba and van der Laan (in preparation) also using the WSRT in redundancy mode, have shown that BL Lac has a symmetric elongated halo extending about 20 arcs in the north–south direction. Of the seven superluminals, only 3C273 remains one sided.

The implications are that the large scale structure cannot be significantly beamed, except for 3C273 where this remains a possibility. If the relativistic beam model correctly describes the mechanism for superluminal motion, our results imply that superluminality may be only a temporary property of radio sources which occurs when the axis of emission rotates or precesses into the line of sight to the observer.

Observations and data reduction

The WSRT was used in redundancy mode¹⁵ for the observations. In this mode all correlations are made between pairs of telescopes in the 14-element array and use is made of the (assumed) identical phases and amplitudes of the redundant baselines to reduce the number of independent errors (unknowns) per hour angle scan to just one gain and one phase error. The amplitude scale of individual scans may change through overall system gain variations (which enter through the normalization in the least-squares fitting for telescope gain errors) or extinction variations. The one remaining error in the phase takes the form of a linear phase slope across the array. Large scale ionospheric and tropospheric effects, which shift a source around in the sky, manifest themselves after the redundancy corrections as a linear phase gradient superimposed, of course, on the true source phases.

Because successive hour angle scans in a regular east–west array such as the WSRT trace out adjacent radial spokes in the UV-plane without any overlap or crossing points, the adjustment of individual scans must be done using a model for the source. This “aligning of scans”¹⁵ usually converges rapidly because it is only very weakly dependent on extended emission.

Any available information about the source from high resolution maps (VLA, MERLIN) is usually included in the initial model. Three of the superluminal sources are at unfavourable declinations ($|\delta| < 6^\circ$) for an east–west array like the WSRT. This has two consequences: (1) the resolution in declination is very poor with a beam of $3.7 \times 3.7 \text{ cosec } \delta$ arc s and (2) at extreme hour angles the telescopes start to shadow each other, and, the signal has to traverse a much larger airmass which noticeably degrades the data. As shown later both these effects have had their influence on the 3C120 map, which contains very extended east–west structure.

The observations were acquired in late December 1981 and early January 1982. Scheduling problems prevented us from obtaining full 12-h tracks for each source. Observing dates and the data obtained are presented in Table 1. For all sources, except NRAO 140, large gaps remained in the UV-plane coverage and the maps were processed using the clean

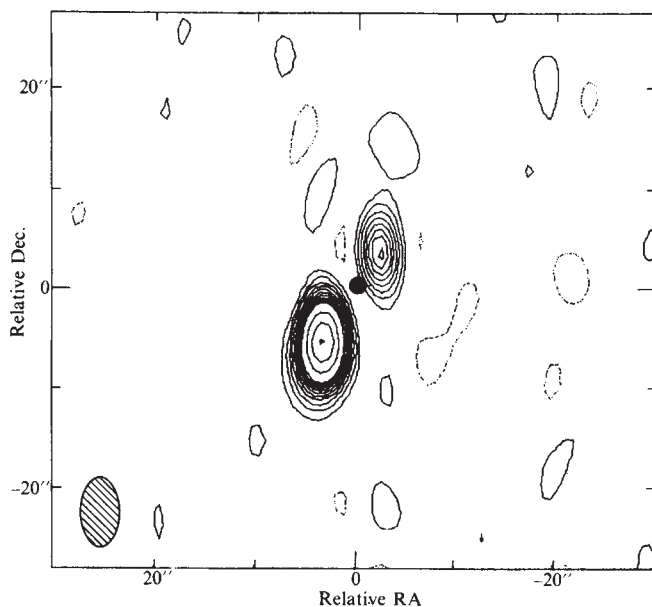


Fig. 1 6-cm map of NRAO 140. The core component (flux density 2,600 mJy) has been removed at the position of the black dot. The contours are $-0.04, 0.04, 0.08, 0.12, \dots, 0.4, 0.6, 0.8$, 1.0% of the core flux density. Dynamic range in the map is limited by thermal noise (0.4 mJy).

Table 1 Summary of observations of superluminal sources

Source	Observing date	Hour-angle coverage (deg)	Total time (h)
NRAO140	1 January 1982	$-82 \rightarrow +90$	11.5
3C120	2 January 1982	$-82 \rightarrow +82$	11
3C273	1 January 1982	$-24 \rightarrow +33$	4
3C279	4 January 1982	$-82 \rightarrow -7$	5
3C345	2 January 1982	$-90 \rightarrow -22$	7.5
		$46 \rightarrow 90$	
3C179	22 December 1981	$-90 \rightarrow +38$	8.5

algorithm; the source components were restored with an elliptical gaussian beam equal in halfwidth to that of the original beam. However, to allow a better view of the emission immediately surrounding the core, these cores were removed from the map by subtracting their response in the UV-plane. The core fluxes were determined from the visibilities on the longest baselines available (about 45,000 λ). Note that the appearance of the map within one beamwidth of the core is strongly affected by the amount of flux subtracted at the core position.

All sources, except 3C179, were observed at 4,875 MHz, with parallel dipoles aligned in position angle (PA) 0° ; in this configuration the array is sensitive to the combination of Stokes parameters $I-Q$. 3C179 was observed at 4,995 MHz, but due to an error the redundant baseline data were not recorded. The flux densities are on the Baars *et al.*¹⁶ scale; they are accurate to $\sim 2\%$. Thermal noise is about 0.5–1 mJy. Because all superluminal sources are linearly polarized, our fluxes, which measure $I-Q$, may be considerably different from those of other observers.

Results

Figures 1–3 display our 6-cm maps of NRAO 140, 3C120 and 3C345. The coordinate scale shown is relative to the position of the unresolved core. Various parameters of the extended components found in the superluminal sources are given in Tables 2 and 3. A search has been made on the Palomar Sky Survey prints for optical counterparts of the extended structure in these sources. With the exception, of course, of the optical jet in 3C273, no convincing counterpart has been found.

NRAO 140 (Fig. 1). The structure consists of three sources of which the central one, the core, was removed from the map.

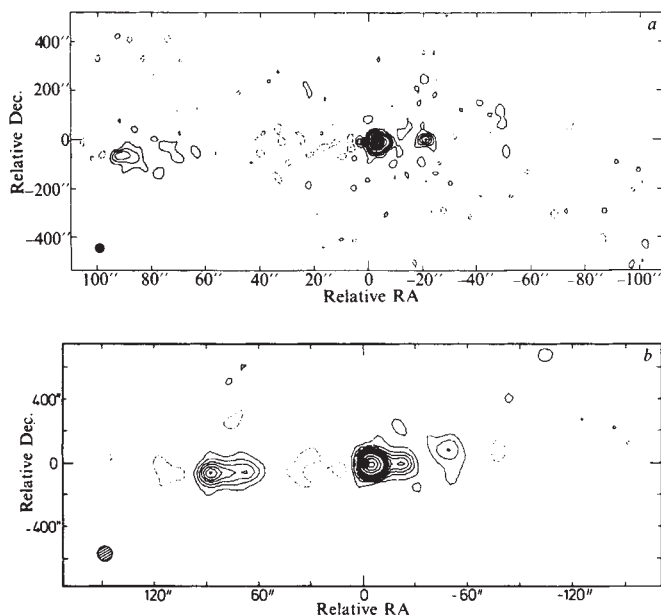
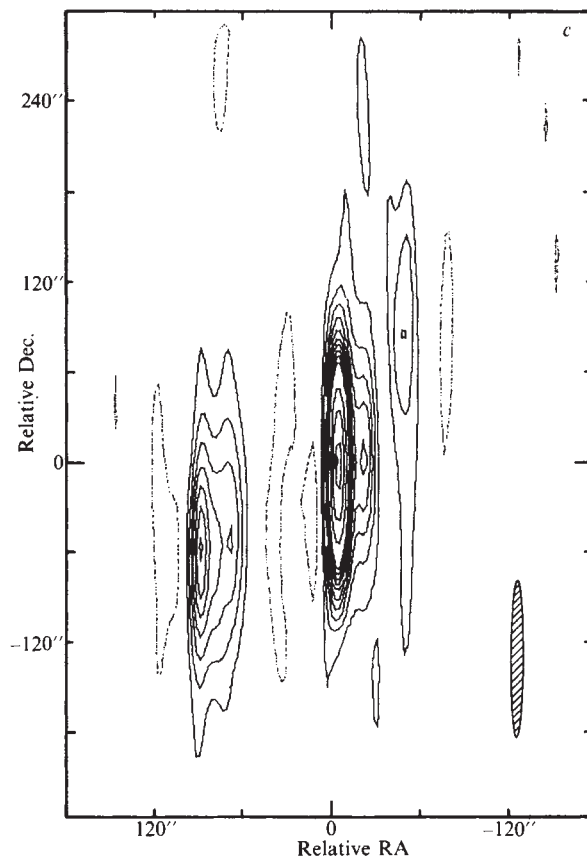


Fig. 2 *a*, 6-cm map of 3C120 at full resolution (3.7 arc s beam) with the declination scale compressed by $1/\sin \delta$. The core component (flux density 2,800 mJy) has been removed at the position of the black dot. Contours are $-0.05, 0.05, 0.10, 0.15, 0.2, 0.25, 0.5, 0.75, \dots, 1.75\%$ of the core flux density. *b*, Shows *a* convolved to a 10 arc s beam. Contours are $-0.1, 0.1, 0.2, 0.3, \dots, 1.0, 1.5, 2.0, 2.5\%$ of the peak flux density of 2,800 mJy. *c*, As for *b* but with equiangular coordinates.



The two outer components appear unresolved. However, the visibility data indicate the presence of some 10 mJy of extended emission on a scale of about 20 arc s; Fig. 1 may show some traces of this low-surface brightness emission. The north-west component was not detected by either the MERLIN 73-cm or the VLA 6- and 20-cm observations^{12,13} presumably because it was below the detection limit, or resolved. The south-east component was detected with all three telescopes, with total flux densities 140 mJy at 73 cm (MERLIN), ~ 35 mJy at 20 cm (VLA) and 27 mJy at 6 cm (WSRT). Perley¹³ did not see the south-east component at 6 cm; his flux density limit of 5 mJy suggests that this component is completely resolved at 0.3 arc s resolution. The 20 cm VLA flux must also be a lower limit. The spectral index of the south-east component as determined from the flux densities at 73 and 6 cm is -0.66 ($S \propto \nu^{-\alpha}$). Although the VLBI data^{17,18} are not complete enough to enable a hybrid map to be made, they do indicate that the m arc s structure is elongated along PA $\sim 130^\circ$, which is $\sim 20^\circ$ from the line joining the outer components in the WSRT map to the core (PA $\sim 150^\circ$).

3C120 (Fig. 2). Extended structure on an arc min scale clearly exists to the east and west of the core component. The jet component mapped by the MERLIN at 73 cm (ref. 12) is seen in the WSRT full resolution map (Fig. 2a) as an extension to the west of the core. Previously unmapped by MERLIN or the VLA (ref. 19) are the compact component ~ 24 arc s west of the core (PA -67°) ((2) in Table 2), the extended component ~ 105 arc s south-east of the core in PA 121° ((3), in Table 2), and the diffuse component ((1) in Table 2) ~ 106 arc s to the north-west in PA -29° (see the convolved maps in Figs 2b and c). We do not find evidence for emission from any counter jet. The small blob just to the east of the subtracted core is at the limit of the map.

Component (2) appears to lie to the south of the extension of the MERLIN jet but this could be the result of poor positional accuracy in declination for the WSRT at $\delta + 5^\circ$. It must, however, be the continuation of the jet. Component (3) is the strongest of all the outlying components, and it lies on the opposite side of the core to the VLBI and MERLIN jets. The

extension of component (3) is well aligned with the axis joining the core to component (3), leaving little doubt that this outlying component is related to 3C120. Component (1) to the north-west appears to coincide with a galaxy close to 3C120 (see Fig. 5 in ref. 20). However, the full resolution map (Fig. 2a) demonstrates that component (1) is very diffuse, quite unlike nuclear radio components in many other galaxies. Moreover 21-cm redundancy maps (in preparation) show this component is extended and is connected to the core of 3C120 by a bridge of emission. Component (1) must therefore also be associated with 3C120. Component (2) lies on the outer edge of the optical image of 3C120 while component (3) to the south-east does not coincide with any optical objects on the appropriate Palomar Sky Survey print.

The negative contours on the eastern side of the core component and component (3) are caused by not correcting properly for the presence of the extended component of which the components (1) and (3) are only the brightest features. This component can only be detected at extreme hour angles when the data are severely disturbed by shadowing problems in the array. Its 6-cm flux density is at least 300 mJy. The extended component shows up much more clearly at 21 cm; at 50 cm it has a total flux density of about 2 Jy (unpublished WSRT data). **3C179.** A map was made in normal mode which confirmed the general morphology found with MERLIN (ref. 12). However, there is a suggestion in our map (which has a dynamic range of only 100) of a 10 mJy component ~ 10 arc s to the west of the western lobe.

3C273. Since 3C273 is at $\delta + 2^\circ$, essentially only one-dimensional information is obtainable with an east-west interferometer. A profile through 3C273 has been made with most of the flux density of the core component and the well-known jet in PA -137° removed to allow a sensitive scale to be used for the counter jet region and the region beyond the jet. There is no evidence of any emission in excess of 20 mJy in either of these regions; a limit of $< 1/300$ can therefore be set for the ratio of the flux density of any counterjet to the jet, in agreement with results from VLA. A discrete source 8 arc min away in PA $-142 \pm 2^\circ$ (ref. 21) is clearly detected in our map with a

Table 2 Parameters of the core and extended components in NRAO140 and 3C120

Source	Component	Offset from core Radius (arc s)	PA (deg)	Flux density ($I-Q$) (mJy)	Component size (arc s)
NRAO140	NW	4.1	-31	9	≤ 2
	Core	0	0	2,600	≤ 0.3
	SE	6.9	149	27	≤ 2
3C120	NW (1)	106	-29	$\gg 5$	$\sim 10 \times < 70$
	W (2)	24	-67	7	≤ 3
	Jet			50	
	Core	0	0	2,800	≤ 0.3
	SE (3)	105	121	> 50	$\sim 35 \times < 70$

Table 3 Flux densities and overall sizes of the cores and extended components in the superluminal sources

Source	Overall size (arc s)	Flux density at 6 cm ($I-Q$, mJy)	
		Core	Extended
NRAO140	11	2,600	36 (NW+NE) ~ 10 diffuse
BL Lac	20	4,500	25
3C120	200	2,800	$\gg 5$ (NW) > 50 (SE) ~ 50 (Wjet)
3C179	15	510	250 (W) 270 (E)
3C273	21	26,000	5,600
3C279	15	10,000	1,300
3C345	20	10,150	300-350 (NWjet) 250-200 (S+NE)

6-cm flux density of about 125 mJy (corrected for a factor 4.5 primary beam attenuation); it is a steep spectrum unequal double with east-west separation of ~ 6 arc s. It is not clear if this source is associated with 3C273.

3C279. The data for this source suffer from poor weather conditions and, moreover, are limited in hour-angle coverage. Our map which has a dynamic range of about 1,000 shows emission to the north-west and south-east in agreement with MERLIN and VLA results^{12,13}. No emission on scales from 20 to 60 arc s has been found in the present observation to a level of 50 mJy in the east-west direction.

3C345. The brightness distribution in Fig. 3 shows discrete emission features to the north-east and south of the core that are not observed with MERLIN (ref. 12). These features appear to lie on top of a more extended 15×20 arc s halo. The existence of such a halo has already been suggested by Browne *et al.*¹² and, in fact, seen by Perley *et al.*²². The structure to the north-west lies approximately in the direction of the 3 arc s jet seen with MERLIN. The detailed intensity distribution in this direction is unknown, and it is this uncertainty, coupled with the effects of the gap in UV-coverage, which has led to the negative hole to the north-west of the jet. Elsewhere in the map, the dynamic range is limited by the thermal noise. No larger scale emission > 30 arc s has been found in the present observations to a limit of 50 mJy. This is confirmed in 21-cm observations (unpublished data). In the latter observations no evidence was found to corroborate the suggestion²¹ for an association between 3C345 and several surrounding discrete sources.

Discussion

Six of the seven superluminal sources have extended structure on the tens of arc s scale on either side of the core component, the exception being 3C273 which is one-sided. Does this property make the superluminal sources different from other core-dominated sources? Indications that this is not the case comes from a VLA study of 404 compact sources by Perley¹³, including all superluminal sources. It appears that the latter as

a group do not differ significantly in their structure from the other 397 sources in Perley's sample. In his sample, diffuse secondary structure was found to be common in core-dominated sources, and in most cases this secondary structure was asymmetrically disposed about the core. Perley further concluded that since the flux ratios for the secondary components are, in general, higher than in classical double radio sources these secondary structures cannot be identified directly with the normal lobes of radio sources. We expect, however, that when observed with lower resolution and/or higher dynamic range many of Perley's¹³ sources will reveal more symmetric extended structures. And these, rather than the compact asymmetric arc s structures, might be identified as the normal lobes of these radio sources. Our result on the superluminal sources is further explored below in the light of this hypothesis.

Figure 4a shows the extended structures of the superluminal sources on the largest angular size-redshift diagram for the 3CR sample of Hooley *et al.*²³. The superluminal extended structures are plotted twice, once assuming they lie in the plane of the sky, and again assuming they lie at the small angle to the line of sight indicated by the superluminal motion. The deprojection factor is taken equal to $\gamma_{\min}^{-1}$ where $\gamma_{\min}$ is the minimum Lorentz factor given by Cohen and Unwin². Assuming the superluminal extended structures lie in the plane of the sky, they occupy median positions in size in the diagram. However, if the angles to the line of sight are those indicated by the superluminal motion, the extended structures of the superluminal quasars, in all cases except 3C273, are either the largest, or close to the largest, sources at those redshifts. For large γ s it may not be correct to deproject the largest angular size by as large a factor as $\gamma_{\min}^{-1}$ due to a combination of the morphology of the extended components and their transverse sizes. This may be relevant for 3C279 and 3C345, but even so, it does not change the conclusions.

Another way of expressing this result is to plot the positions of the superluminal quasars on the luminosity-size diagram for 3CR quasars²³. Their luminosities at a rest frequency of 178 MHz have been calculated using measured flux values of the extended components at low frequencies (for example, 610 MHz) or from the present 6-cm flux densities assuming a spectral index of -0.8 (see Table 3). In the 3CR sample, the median angle to the line of sight of the extended structure is expected to be 60° . Accordingly we have decreased the deprojected sizes of the superluminal quasars by a factor of 1.15 to be consistent, in a statistical sense, with the sizes plotted for the other sources in the diagram. There are selection effects arising from the flux-limited sample which govern the distribution of low luminosity quasars in the diagram, but it is clear that the probability of the superluminals being the largest sources, if drawn at random from the quasar population, is very small. One can conclude that the extended structures of the superluminal sources do not lie closer to the line of sight to the observer than about 30° .

It is important to stress that the dynamic range in our maps does not differ greatly from that in the Cambridge maps on which Fig. 4a and b are based, in so far as the extended structure is concerned. Therefore it is legitimate to include our values of luminosity and size in these. Note that WSRT redundancy measurements (A. G. de B., in preparation) on some of the unresolved sources in these diagrams (such as 3C48, 3C147 and 3C286) as well as recent MERLIN maps (3C309.1, 3C380; ref. 24) reveal low level extended structure around the dominant core components. The true positions of these five objects on diagrams such as Fig. 4a and b are near the median. One must, however, mention that if the cores in these five sources have not been Doppler-boosted as a result of a favourable orientation, then the dynamic range in these maps is much larger than that in the maps from which the entries in Fig. 4a and b were taken, and the comparison may not be fair.

Figure 4b shows that the extended structures of the superluminal quasars occupy the lower luminosity part of the diagram. On the relativistic beaming hypothesis, this can be

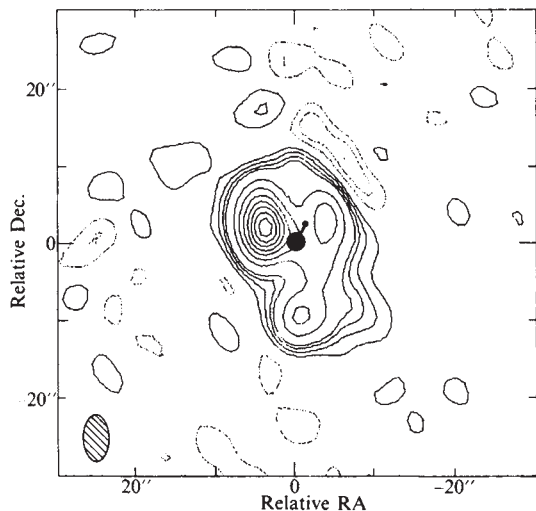


Fig. 3 6-cm map of 3C345. The core component (flux density 10,150 mJy) and the arc s jet (flux density 350 mJy) have been removed from the map as indicated. Contours are -0.025, -0.0125, 0.0125, 0.025, 0.0375, 0.05, 0.1, 0.15, ..., 0.4% of the core flux density.

understood by supposing that the superluminals are representative of a large population of quasars with a broad luminosity distribution, and have only reached our attention because the core flux density has been boosted sufficiently to allow inclusion of the sources in flux limited samples like the 3C catalogue. In fact, 3C120 and 3C345 do not qualify for the 3C catalogue on the basis of their extended emission. About 70% of the extended quasars in Fig. 4b are lobe-dominated at frequencies below 1 GHz. No boosting of the core flux density greater than a factor ~ 20 will occur for angles to the line of sight $\geq 20^\circ$ (Fig. 7b in ref. 1). We expect two of the extended quasars to lie by chance within 20° of the line of sight, and there are eight candidates with strong cores.

Are the extended structures moving relativistically? The flux ratios that we and others observe ($< 5:1$; see Tables 2 and 3) suggest that v/c could still be ~ 0.5 if the angle of the source axis to the line of sight is $\leq 40^\circ$ (Fig. 7b in ref. 1). However, in the case of the south-east component of 3C120 which is the strongest outlying component in the source, we need to postulate that it is moving relativistically towards us in order to Doppler-boost its flux density, whereas the orientation of the VLBI and MERLIN jets is to the west implying that the south-east component is most likely receding. It is, therefore, unlikely that the extended structure moves relativistically.

What implications do these conclusions have for the relativistic beaming model for superluminal motion? First, the axis of ejection for the m arc s structure cannot be aligned with the axis of the extended structure. If the angle to the line of sight of the m arc s structure were as large as 30° , superluminal motion could still be observed for highly relativistic bulk motion, but only to a maximum value of ~ 4 times the speed of light (see Fig. 7a in ref. 1). The observed γ s are all greater than, or equal to, this value (for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$). We are therefore forced to decouple the orientations of the compact and extended structure.

Rees²⁵ has suggested that relativistic material could be ejected over a range of angles around the symmetry axis of the source, some of which are likely to be close to the line of sight. Superluminal motion would then be observed even though the bulk of the emission is directed at a substantial angle to the line of sight. We feel this concept is unattractive for two reasons. First, the motion of the emitting material must always be highly relativistic and within a few degrees of the line of sight to boost the 'sidelobe' emission sufficiently that it dominates over the main discharge and becomes the only emission seen in present day VLBI maps with dynamic range 100–1. Second, it seems unlikely that there are no other sidelobes which are directed close to the line of sight. This implies that multiple beams with

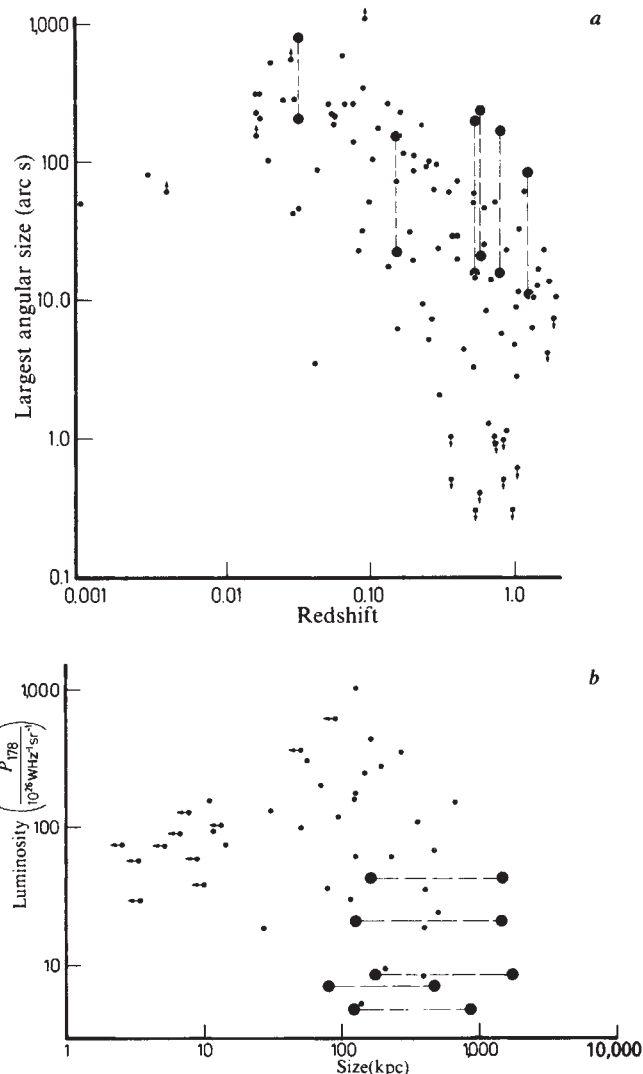


Fig. 4 a, Largest angular size plotted against redshift for the 3CR sample, adapted from ref. 23, with superluminal sources marked (see text). b, Luminosity-size diagram for the 3CR quasars, adapted from ref. 23, with superluminal sources marked (see text); $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, $q_0 = 0$.

different γ s should be visible on m arc s scales in present day maps, which is not observed to be the case.

We prefer to consider the possibility of the current axis of ejection precessing or wobbling into the line of sight, thereby producing superluminal motion for a relatively short period. Precession effects, or wobbles of the fundamental axis, are not uncommon in lower luminosity radio galaxies²⁶. Such effects are also to be seen in about 30% of the 3CR quasars in Fig. 4a. Typical precession periods are thought to be 10^6 – 10^7 yr. We envisage the extended structure in the superluminals seen with the WSRT as the oldest remnants of emission, and the arc s jets seen with MERLIN and the VLA as more recent emission produced as the axis of ejection moved towards its present position close to the line of sight.

According to Linfield²⁷, precession with periods of the order of 10^3 yr, cone half-angles of a few degrees, and precession axis close to the line of sight, could explain the large changes observed in the orientation of the m arc s radio structure in core-dominated sources (see, for example, ref. 6). The same precessional parameters could not be made to apply to the arc s structure because this is not sufficiently curved. Two consequences of precession on the m arc s scales are that the angle to the line of sight, and the position angle of the structure projected on the sky can change as a function of period. The former will lead to a change in the apparent superluminal

velocity of the ejected matter. There is some evidence of both effects in 3C345 (ref. 28), and in 3C120, of a change in the apparent superluminal velocity which is not accompanied by a position angle change (R. C. Walker, personal communication). These results are not inconsistent with precession models.

In our general picture of the superluminal sources, this short precession or wobble would be superimposed on the much longer precessional motion underlying the structures observed with the WSRT.

The 'unified' scheme of Orr and Browne²⁹, itself an expansion of an earlier scheme by Scheuer and Readhead⁹ and Readhead³⁰, suggests that core-dominated sources are steep spectrum doubles seen end-on. The observed differences between the two types of source are attributed to the effects of relativistic beaming and projection. The relative numbers of core-domi-

nated and steep spectrum objects can be made consistent with the scheme if $\gamma_{\text{core}} \sim 5$ (see also ref. 31). Our data give direct evidence that a large scale extended structure exists associated with the core sources expected in the unified scheme. Furthermore, there is no obvious effect on the statistics of the unified scheme of incorporating a long-term precession, as one expects the precession axes to be randomly distributed in space.

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Movement of myosin-coated fluorescent beads on actin cables *in vitro*

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Myosin-coated fluorescent beads are observed to move unidirectionally along organized actin filament arrays in the alga, Nitella, with an average velocity similar to in vivo rates of movement in muscle and other cells. The myosin produces the motive force, as the movement is ATP dependent and is blocked by inactivation of the myosin heads. The movement, which occurs in the absence of bipolar thick filaments, provides a quantitative assay for myosin motility.

MUSCLE contraction and other forms of cell motility are believed to be driven by myosin molecules pulling themselves along actin filaments. Indeed, the classic studies of Huxley (for review see ref. 1) have shown that myosin filaments slide past actin filaments during the shortening of the sarcomere. Similar sliding of myosin over actin is believed to provide the motive force for most cellular motile activities such as cytoplasmic streaming, cell locomotion and cell division. Although the understanding of ATP-dependent movement of myosin along actin filaments is paramount to the understanding of motility, the molecular basis of myosin movement remains obscure. There has been no direct visualization of myosin molecules moving and thus no direct assay has yet been devised for this myosin movement. Such an assay is essential to an examination of the biochemical and biophysical requirements for this energy transduction process.

The sliding filament theory of muscle contraction proposed by Huxley and Hanson² and Huxley and Niedergerke³ is now well established, and many studies of muscle structure as well as the molecular architecture of myosin⁴ suggest that force generation may be the result of a change in the orientation of myosin heads while attached to actin filaments^{1,5}. Myosin heads bind to actin filaments with a very specific orientation in an

ATP-dissociable manner⁶. Two recent studies that measure changes in myosin heads, referred to as cross-bridges in muscle, are consistent with a change in orientation during muscle contraction. Huxley *et al.*⁷ have obtained millisecond time-resolved changes in appropriate X-ray reflections during rapid mechanical transients, and Borejdo *et al.*⁸ found fluctuations during contraction in the polarization of the fluorescence of a probe attached to the myosin head. In the proposed moving cross-bridge mechanism, an ATP-induced dissociation of actin and myosin allows many repetitive interactions⁹, leading to the 'walking' of myosin heads along actin filaments. There are various possible alternative explanations for the mechanism of force generation. For example, Harrington¹⁰ has proposed that the motive force may originate from a helix-coil transition in the hinge region of the myosin tail. Yanagida¹¹ has emphasized that changes in the actin filament, rather than in the myosin, may be responsible for movement.

An important implication of all of these mechanisms is that actin and myosin are alone sufficient for movement. The sarcomere contains a variety of accessory proteins whose roles are not completely understood, and it has therefore been critical to obtain reconstituted systems that show movement where the minimal number of components required could be determined.

Table 1 Requirements for HMM-bead movement

Pretreatments		ATP concentration (mM)	Total no. HMM-beads	% Moving HMM-beads
HMM-beads	<i>Nitella</i>			
(1) DTT then NEM	None	1	110	83
(2) NEM then DTT	None	1	54	0
(3) None	None	0	89	0
(4) Washing	None	1	109	42
(5) None	NEM then DTT	1	88	90

HMM-beads were applied to the cytoplasmic face of *Nitella* and monitored for 10–30 min on video tapes. Only those HMM-beads which became attached to the substratum, as evidenced by the cessation of brownian motion, were scored. (1) HMM-beads ($\sim 10^9 \text{ ml}^{-1}$) were treated for 1 h on ice with 2 mM DTT followed by incubation in 1 mM NEM. (2) HMM-beads were treated for 1 h on ice with 1 mM NEM followed by incubation in 2 mM DTT. (3) ATP was omitted from all of the buffers in this experiment. (4) The HMM-bead preparation was washed three times by sedimentation for 1 min at 13,000g and resuspension in 50 mM KCl, 10 mM potassium phosphate, pH 7.0, 0.2 mM DTT. The HMM used in these experiments hydrolysed ATP in 10 mM Tris, pH 7.7, 0.5 M KCl, 2 mM EDTA at 25°C at a rate of 0.24 $\mu\text{mol P}_i$ per min per mg HMM. The units of HMM ATPase activity in the third supernatant were only 10% of the activity in the corresponding HMM-bead pellet, which had an activity of $8.4 \times 10^{-12} \mu\text{mol P}_i$ per min per bead (the concentration of beads was determined by counting the HMM-bead suspension in a haemocytometer after sonication to disrupt bead aggregates). This value corresponds to $3.5 \times 10^{-11} \text{ mg HMM per bead}$ or 6×10^4 molecules of HMM per bead. As the beads are 0.7 μm in diameter, there is 1 myosin head for every $\sim 12 \text{ nm}^2$. The dimensions of a myosin head³¹ are about $3 \times 8 \times 12 \text{ nm}$. (5) The intact *Nitella* was treated with 1 mM NEM for 20 min, by which time all cytoplasmic streaming had stopped. The *Nitella* was then dissected into buffer A (see Fig. 1 legend) with 1 mM DTT and HMM-beads were added.

Accordingly, several *in vitro* movement systems have been developed, some of which have made use of demembranated cytoplasmic droplets from the giant cells of algae such as *Nitella* (for review, see ref. 12). Thus, Kuroda and Kamiya¹³ described the rotation of chloroplasts in such droplets. When *N*-ethylmaleimide (NEM) was added, the chloroplast movement stopped, but a slow and sporadic rotation resumed when heavy meromyosin (HMM), a fragment of myosin lacking a portion of the myosin tail, was added in the presence of Mg^{2+} and ATP. More recently, Higashi-Fujime¹⁴ examined cytoplasmic droplets and observed movement of fibrils as well as chloroplast chains linked together by fibrils. These fibrils were shown to contain F-actin and moved at about $10 \mu\text{m s}^{-1}$. *Nitella* myosin, which has been isolated and characterized by Kato and Tonomura¹⁵, is assumed to produce the active shear force responsible for these *in vitro* movements of actin-containing structures (see ref. 12). Such studies provide further evidence for the roles of actin and myosin but are nonetheless complicated by the presence of many cytoplasmic components. Other *in vitro* motility systems have been devised that are totally defined biochemically. In one case Yano¹⁶ and Yano, Yamada and Shimizu¹⁷ observed streaming between two concentric cylinders coated with F-actin. The streaming occurred at $20 \mu\text{m s}^{-1}$ and required Mg^{2+} , ATP and HMM. In another case, Yano, Yamamoto and Shimizu¹⁸ observed the rotation of a rotor when one surface of each blade was coated with F-actin and the rotor was suspended in a solution containing Mg^{2+} , ATP and HMM. The movement was always in one direction and rotations of 10–50° were reported. It can be concluded that the only two proteins required for these movements are actin and myosin.

To pursue the analysis and determine the molecular mechanism of force generation by these proteins, a rapid quantitative assay of movement is required. We have now devised such an assay, based on direct visualization of myosin movement along an actin substratum.

Experimental measurement of myosin movement

No method has been devised for measuring myosin movement because of the difficulties of obtaining a fixed and organized actin substratum and of monitoring the position of myosin. In the giant internodal cells of *Nitella*, polar cables of actin filaments are fixed on the cytoplasmic face of rows of chloroplasts, which line the walls of the cells. There are about five

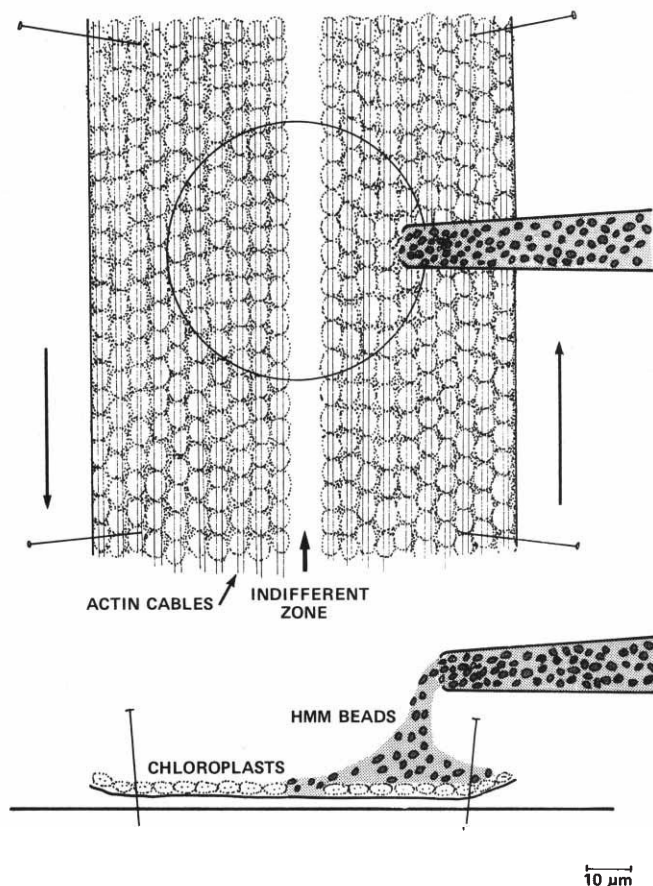


Fig. 1 Schematic drawing of the experimental procedure for assay of myosin movement, showing HMM-beads added to *Nitella* actin filaments exposed by dissection of the cell. An individual cell of *Nitella* is immersed in buffer A (25 mM KCl, 4 mM MgCl_2 , 4 mM EGTA, 5 mM imidazole, 1 mM ATP, pH 7.0), cut along its long cylindrical axis with microscissors, and pinned at the edges to a silicone substratum in a Petri dish with the cytoplasmic face of the cell membrane exposed. The large circle in the top view shows the approximate field of view with a water-immersible $\times 40$ objective (Zeiss). All the filament bundles have one polarity to the left of the indifferent zone and the opposite polarity to the right as indicated by the long thin arrows. HMM, prepared by proteolytic digestion of rabbit skeletal muscle myosin⁴, is reacted with fluorescent Covaspheres (Covalescent Technologies Inc., Ann Arbor). Reaction conditions are 0.4 to 4.0 mg ml^{-1} of HMM in 0.05 M KCl, 0.2 mM DTT and 10 mM potassium phosphate, pH 7.0, with a 1:9 dilution of the stock Covaspheres, at 0°C for at least 2 h. These HMM-beads are diluted 1:3 in buffer B (4 mM MgCl_2 , 4 mM EGTA, 0.2 M sucrose, 0.8% polyethylene glycol, 25 mM KCl, 50 mM Tris, 1 mM ATP, pH 7.6)¹⁴ and pipetted on to the *Nitella* cytoplasmic surface. The HMM-beads then settle on the rows of actin cables.

cables per row of chloroplasts¹⁹, and each cable is about 0.2 μm in diameter and consists of hundreds of actin filaments all of the same polarity. The polarity of the actin filaments is such that a myosin molecule would be expected to move in the direction of the *in vivo* cytoplasmic streaming²⁰. We have used these well organized polar arrays of actin filaments as an actin substratum. To monitor the movement of myosin molecules along these actin filaments, we have constructed HMM-beads by covalently linking rabbit skeletal muscle HMM to the surface of 0.7 μm fluorescent beads.

The experimental procedure consists of cutting open a large ($\sim 1 \text{ mm} \times 4 \text{ cm}$) cell of *Nitella axillaris* and pinning it down so that the cytoplasmic surface faces a water immersion microscope objective (Fig. 1). The cytoplasm is washed away, exposing the cytoplasmic surface of the chloroplast bed. Fluorescent beads, 0.7 μm in diameter and coated by covalent linkage of skeletal muscle HMM, are applied and observed in the microscope. Numerous useful fields are obtained from a single cell

and can be used over several hours. When a cell is cut open into a medium containing Mg^{2+} and ATP, motile elements of the remaining cytoplasm stream off the edges of the dissected cell. Thus, an actin substratum freed from endogenous motile elements is obtained such that motile activity of the HMM-beads can be observed *in vitro*.

Myosin-bead movement

A striking directed movement of HMM-beads (single HMM-beads or aggregates of HMM-beads) occurs after they settle on the actin substratum and attach, ceasing brownian motion. Time-lapse photographs show that the movement is rapid and directed along chloroplast rows (Fig. 2). No tumbling or major reorientation is necessary for motion, as linear bead aggregates maintain a stable orientation during translation (Fig. 2d). The HMM-beads always move along a row of chloroplasts even when the chloroplast row assumes a curved path (Fig. 2e; see also bead 6 in Fig. 4). Occasionally, bulk fluid flows are observed, due to convection or the force of application of the beads. Bead movement in bulk fluid flow is characterized by an increased velocity with increased distance from the chloroplast layer, brownian motion of the beads, and the movement of all particles in the field which exhibit brownian motion. Thus, the directed movement of HMM-beads along chloroplast rows is very distinct from bulk fluid movements.

Although the directed movement of HMM-beads along chloroplast rows is rapid, it often involves several changes in velocity before the particle stops and becomes fixed on the chloroplast layer. The maximum velocity for each HMM-bead was obtained from video recordings of the movement. A total of 74 HMM-beads were analysed and the distribution of maximum velocities is shown in Fig. 3. The velocities range over $0.5\text{--}10\ \mu\text{m s}^{-1}$ (average $2.5\ \mu\text{m s}^{-1}$); these are comparable with the rates of relative sliding of myosin and actin filaments in muscle. Included in this analysis are aggregates as large as $\sim 10\ \mu\text{m}$ in diameter as well as single $0.7\ \mu\text{m}$ beads.

Direction of movement

An advantage of *Nitella* is that the actin filaments occur in two groups with opposite polarity. These groups are separated by an indifferent zone, defined as a region on the cytoplasmic face of the cell membrane that is relatively free of filaments and rows of chloroplasts (see Fig. 1). On one side of the indifferent zone all of the actin filaments have the same polarity, as shown by their decoration with myosin heads to give the actin filaments an arrowheaded appearance⁶, and the direction of *in vivo* cytoplasmic streaming is opposite to that of the arrowheads²⁰. On the other side of the indifferent zone, both the polarity of the actin filaments and the direction of cytoplasmic streaming are reversed. As shown in Fig. 4, HMM-beads observed *in vitro* on both sides of the indifferent zone move in opposite directions, and the directions are the same as those of the cytoplasmic streaming *in vivo* (observed in the intact cell before dissection). Because the HMM-beads settle on the chloroplast layer at different times, at most only a few particles in a field move at the same time. The six HMM-beads shown in Fig. 4 were not all moving simultaneously but were observed over a 10-min period. Because the HMM-beads move in opposite directions on opposing sides of the indifferent zone, the motive force clearly is provided by the HMM-beads themselves and not by fluid flow. Thus, movement of beads occurs with the expected characteristics of a myosin-driven process; it requires contact of the beads with the actin cables and proceeds in the proper direction.

Conditions for movement

If the HMM-bead movement is driven by the myosin head groups, ATP should be required and specific inactivation of the myosin heads should block movement. Table 1 shows that ATP is indeed necessary for HMM-bead movement. To inactivate the myosin heads specifically, the HMM-beads are treated with NEM, which is known to react with critical sulphhydryl groups

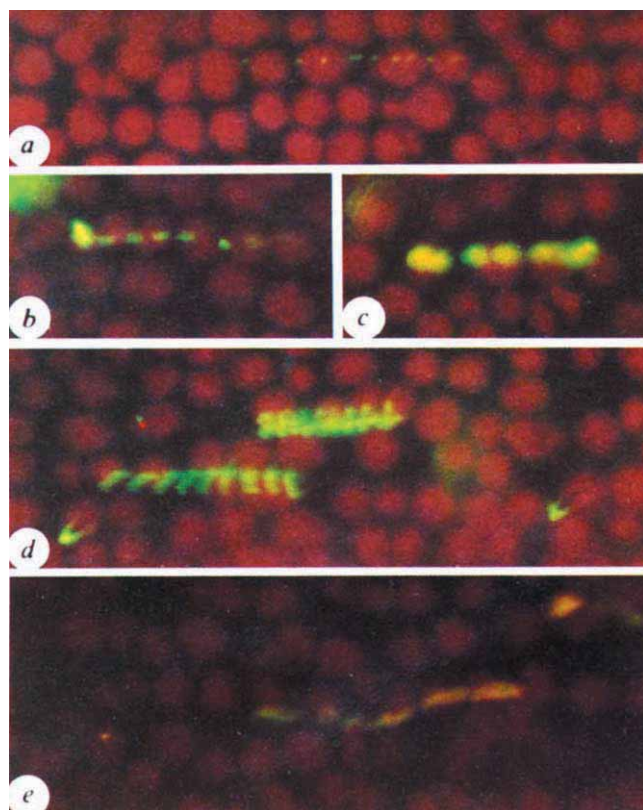


Fig. 2 Light micrographs of HMM-beads moving along the chloroplast rows. Each photograph was produced by a series of 1-s exposures (taken every few seconds; total exposure ~ 10 s). *a*, A single HMM-bead moving; *b*, an aggregate of two or three beads; *c*, a larger globular aggregate; *d*, two linear aggregates of three to four beads each; *e*, a globular aggregate which follows chloroplast rows through a curve. $\times 1,000$.

on each myosin head^{21,22}. NEM-HMM will bind to actin but will not dissociate with Mg^{2+} and ATP and will therefore not produce a contractile force²³. After reaction with 1 mM NEM for 1 h followed by treatment with 2 mM dithiothreitol (DTT) to inactivate the residual NEM, the HMM-beads are no longer motile (Table 1). If the HMM-beads are incubated with 2 mM DTT before the addition of 1 mM NEM, normal motility is observed. Although beads coated with bovine serum albumin were tested and found not to move (data not shown), the NEM inactivation of myosin provides a better control for nonspecific causes of motion. The only difference between the moving and non-moving HMM-beads is the presence of the alkylating agent on the critical sulphhydryl groups of the myosin.

NEM is also known to inactivate the endogenous motile components in the *Nitella* cytoplasm²⁴ that could conceivably contribute to bead motion *in vitro*. Abolition of streaming in *Nitella* by treatment with NEM before dissection does not affect HMM-bead movement on that *Nitella* actin substratum (Table 1).

Some free HMM is added with the HMM-bead preparations but it is unlikely to contribute to bead movement. First, the hydrodynamic considerations of Nothnagel and Webb²⁵ indicate that free HMM should not cause streaming in this situation, where the actin cables cover only a small fraction of the surface area of the *Nitella* substratum used. Second, the characteristics of bead movement are inconsistent with general streaming, in that normally only one or two beads are moving in a field at a given time. Finally, this possibility was tested further by washing the HMM-beads free of unbound HMM; motility of the HMM-beads, which cannot now be explained by any trace of free HMM remaining, is still observed. There is, however some reduction of motile activity after washing the beads which is as yet unexplained. The number of bound HMM molecules per bead (6×10^4), determined by ATPase analysis of washed bead

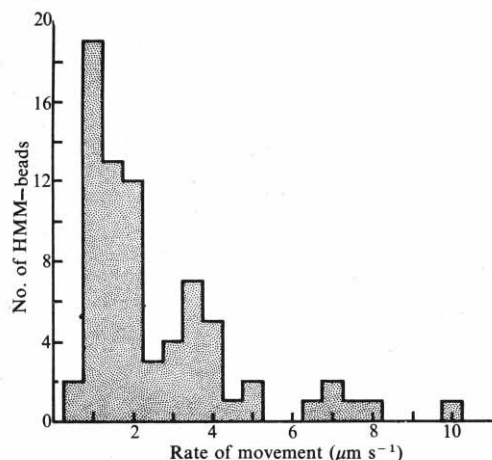


Fig. 3 Histogram relating the number of moving HMM-beads analysed and their rates of movement. On average, each of the 74 HMM-beads (single beads and distinct bead aggregates) was followed for about 40 μm . The rate of movement of a given HMM-bead was variable over that distance (for example, see Fig. 4); the maximum rate of migration of each particle measured over at least a 10- μm distance is plotted.

preparations, is sufficient to form a close-packed layer on the entire bead surface (Table 1).

As expected, the HMM-bead movement does not require Ca^{2+} , as cytoplasmic streaming in *Nitella* is not Ca^{2+} -dependent¹². In *Nitella*, unlike in skeletal muscle, the actin filaments are not under Ca^{2+} control.

All of these observations on the requirements for HMM-bead movement indicate that HMM attached to the bead surface is responsible for the observed movement and that the HMM must be capable of going through its association-dissociation cycle with actin.

Energetics

In the motility system described here, HMM-beads move at quite rapid rates, and important inferences can be derived from measurement of these velocities. First, the energy expenditure required to move a single bead at about $5 \mu\text{m s}^{-1}$ is quite small. The drag force (F) on the bead can be computed from $F = 6\pi\eta rv$, where η is the viscosity, r the bead radius and v the bead velocity. For $\eta = 0.01 \text{ P}$, $r = 0.35 \times 10^{-4} \text{ cm}$ and $v = 5 \times 10^{-4} \text{ cm s}^{-1}$, the drag force is $3 \times 10^{-9} \text{ dyn}$. The energy expenditure required to move a single bead at $5 \mu\text{m s}^{-1}$ can then be calculated to be $1.5 \times 10^{-12} \text{ erg s}^{-1}$. As the maximum rate of actin-activated HMM ATPase is about 40 mols ATP per mol HMM per s, about $1 \times 10^{-11} \text{ erg s}^{-1}$ could be provided by a single HMM molecule²⁶. In other words, a single HMM molecule could provide more than enough energy to move an HMM-bead at a velocity of $5 \mu\text{m s}^{-1}$.

The second inference of this velocity of movement comes from estimates²⁷ that the effective stroke distance of a single myosin head is about 100 Å. Using this value, about 500 strokes would be required to move the bead $5 \mu\text{m}$ (because the particle is small, there should be no inertial movement²⁸). Assuming that the maximum turnover rate measured for the actin-activated HMM ATPase reflects the complete stroke cycle, each head undergoes about 20 cycles s^{-1} . Therefore, there must be at least 25 myosin heads contributing to bead movement at a velocity of $5 \mu\text{m s}^{-1}$. On the basis of ATPase assays we determined that there are about 25 functional myosin heads per 300 nm^2 of bead surface. A considerably larger area could easily be in close contact with an individual actin cable which is 200 nm in diameter. Obviously, all of the myosin heads are not expected to be oriented in the proper direction; but even if only 10% are, a reasonable estimate of the area of contact still indicates that the necessary number of myosin heads would be present. More detailed analyses of this point must await data on the geometry of the contact region and on titration experiments to

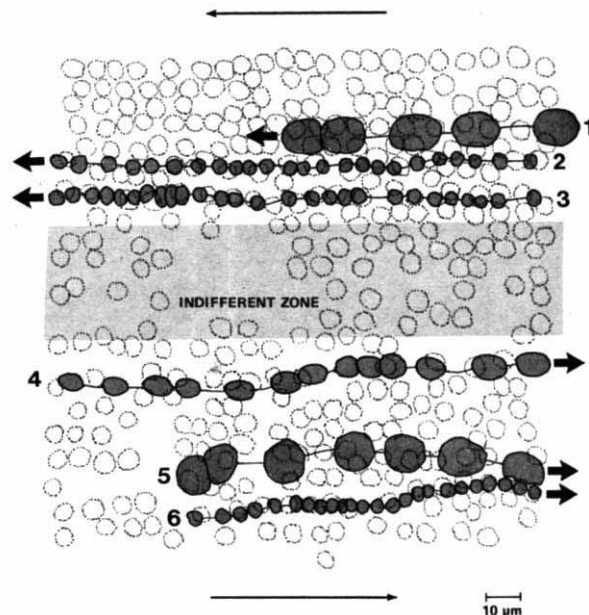


Fig. 4 Opposite direction of movement of HMM-beads on either side of the indifferent zone. The positions of the chloroplasts (dotted circles) and the six HMM-beads (that is, distinct bead aggregates) were determined directly from the video monitor equipped with digital time readout by tracing the particles onto Saran Wrap. The positions of the chloroplasts remained fixed while the HMM-beads moved in the directions shown by the heavy arrows. The long thin arrows indicate the direction of cytoplasmic streaming observed *in vivo* before dissection. The positions of the HMM-beads are shown every 5 s for beads 2, 3 and 6, every 10 s for bead 4 and every 20 s for beads 1 and 5.

determine the minimum average number of myosin molecules per bead required for bead movement. A third inference that follows from the previous two is that larger HMM-bead aggregates could move at similar rates to small ones. This comes from the fact that movement at these velocities consumes much more energy from ATP hydrolysis than is dissipated by fluid drag on a single bead, that is, the force of movement far exceeds that of viscous drag. Indeed, we do observe that large bead aggregates (3–20 μm in diameter) are capable of moving at high rates.

Implication

A central tenet of the swinging cross-bridge model of muscle contraction¹ as well as of non-muscle motility²⁹ is that myosin molecules can walk along actin filaments. We report here the first direct visualization of myosin movement on actin. From a biochemical and structural point of view, we can suggest that myosin need not be in the form of a thick filament in order to move along F-actin. Our HMM preparations contain only small amounts of contaminating myosin (<1%) but produce bead movement at a rate similar to that produced by intact myosin (M.P.S. and J.A.S., unpublished results). More detailed studies are under way to determine the minimum peptide unit which will produce movement, particularly with regard to S1 (subfragment 1 of HMM, a single head of myosin devoid of the entire tail portion).

The orientation of myosin heads on the surface of the HMM-beads is likely to be random. This would imply that only the actin substratum need be oriented to produce directional movement. Because the myosin head binds to the polar actin filament in a precise orientation⁶, presumably those myosin heads that are not oriented properly to contribute to the movement will not bind and will therefore not interfere with the process. Thus, although the high degree of organization of both actin and myosin found in the muscle sarcomere gives maximum efficiency of actin-myosin interaction, it may not be required for movement. It is possible, however, that a nonrandom orientation

could originate from cooperative interactions during the covalent linkage of the HMM to the bead surface. This could produce some local regions of order to the head orientations, but there is no evidence for this. These considerations will require further studies on the structure of the bead surface and of the interface of the HMM-bead with the actin cable.

Because myosin head groups are capable of moving bead aggregates as large as 20 μm in diameter, they should also be capable of moving similarly sized cellular vesicles. The analysis of Nothnagel and Webb²⁵ indicates that linear transport of such structures is necessary to produce the streaming in *Nitella*, and Kersey and Wessells¹⁹ have observed vesicles attached to the actin cables in *Nitella*. Perhaps more important is the fact that such a mechanism could explain many other motile phenomena. For example, vesicular structures in nerve axons move at similar velocities and with similar characteristics to the HMM-bead movements described here³⁰. In general, then, the attachment

of myosin to cellular structures could result in their rapid and directed movement.

Previously, investigators have correlated the actin-activated ATPase activity of myosin with motility. With our *in vitro* assay for myosin movement it is now possible to determine what regulatory factors influence myosin motility *per se*, irrespective of their effects on ATPase activities. Additionally, the regions of the myosin molecule necessary for motility can be mapped. Thus, the development of this assay provides the basis for a detailed analysis of the biochemical and structural features of myosin that allow it to act in the conversion of chemical energy into the mechanical force of movement.

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Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9 Å resolution

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The influenza virus neuraminidase glycoprotein is a tetramer with a box-shaped head, 100×100×60 Å, attached to a slender stalk. The three-dimensional structure of neuraminidase heads shows that each monomer is composed of six topologically identical β -sheets arranged in a propeller formation. The tetrameric enzyme has circular 4-fold symmetry stabilized in part by metal ions bound on the symmetry axis. Sugar residues are attached to four of the five potential glycosylation sequences, and in one case contribute to the interaction between subunits in the tetramer.

TWO glycoproteins are embedded in the membrane of influenza virus, a neuraminidase and a haemagglutinin¹. Haemagglutinin mediates attachment of the virus to host cells², via a sialic acid-containing glyco-conjugate receptor, and the subsequent fusion of viral and host cell membranes^{3,4}. Several roles have been suggested for the neuraminidase (acylneuraminyl hydrolase, EC 3.2.1.18). The enzyme catalyses cleavage of the α -ketosidic linkage between terminal sialic acid and an adjacent sugar residue^{5,6}. This reaction permits transport of the virus through mucin and destroys the haemagglutinin receptor⁷ on the host cell, thus allowing elution of progeny virus particles from infected cells⁸. The removal of sialic acid from the carbohydrate moiety of newly synthesized haemagglutinin and neuraminidase is also necessary to prevent self-aggregation of the virus⁹. In general, then, the role of neuraminidase may be to facilitate mobility of the virus both to and from the site of infection.

Two distinct patterns of antigenic variation have been identified in both neuraminidase and haemagglutinin¹⁰. Epidemics of influenza are associated with minor changes in the structure of the proteins (antigenic drift) and result from point mutations in the viral genome¹¹. Pandemics result from substantial alterations in the chemical structure of the protein (antigenic

shift) and are caused by replacement of the entire gene for haemagglutinin, or neuraminidase, by genetic reassortment^{10,11} or some other mechanism. The amino acid sequence homologies within and between subtypes are about 90% and 50%, respectively, for both haemagglutinin¹² and neuraminidase^{13,14}. Two serologically distinct subtypes of neuraminidase are known in humans¹⁵. The N1 subtype was associated with virus isolated between 1933 and 1957, after which time the N2 subtype appeared in Asian influenza. No major change in the structure of the neuraminidase has occurred since, although the haemagglutinin subtype changed from H2 to H3 in 1968 in the Hong Kong pandemic.

The neuraminidase of influenza virus is an integral membrane glycoprotein. It is a tetramer of molecular weight 240,000, reducing to 200,000 when solubilized from the virus with pronase¹⁶. Sequences of neuraminidases from several strains of N1^{17,18}, N2^{13,14,19–21} and B²² subtypes are known. A hydrophobic N-terminal region serves to anchor the neuraminidase in the membrane^{14,17}. This orientation is uncommon though not without precedent¹⁷. The N2 enzyme subunit contains 469 amino acids, with carbohydrate attached in four places¹⁴. The crystallizable product of the pronase digestion begins at residues 74 or 77¹⁴. These so-called neuraminidase 'heads' carry the full

antigenic and enzymatic capability of the membrane-associated neuraminidase²³.

We describe here the structure of the neuraminidase as determined from an electron density map at 2.9 Å resolution. Two different crystalline N2 neuraminidases, isolates from 1957 and 1967, have been used to determine the structure by a combination of multiple isomorphous replacement and non-crystallographic symmetry averaging methods. The resulting electron density distribution identifies the chain folding, the subunit interfaces and the glycosylation sites. The structure of the enzyme active site and the location of strain-variable segments implicated in the binding of antibodies are discussed in the accompanying article²⁴.

Crystallization

Neuraminidases from two different field strains, A/Tokyo/3/67 and A/RI/5⁺/57, hereafter referred to as Tokyo and RI/5⁺, respectively, have been used in this study. The crystallization and low resolution electron density map of the Tokyo enzyme have been described previously^{25,26}. The space group is I422, $a = 139.6$ Å, $c = 191.0$ Å, with one subunit per asymmetric unit. The RI/5⁺ enzyme was solubilized from X-7F1 virus²⁷ with pronase²³ and crystallized by vapour diffusion against 0.75 M sodium citrate at pH 7. The crystals are octohedral bipyramids with space group P4₂2₁ or enantiomorph, $a = 124.1$ Å, $c = 181.2$ Å and two subunits per asymmetric unit. Both crystal forms diffract X rays at Bragg spacings of 3 Å.

Structure determination

Tokyo/3/67 neuraminidase: Tokyo neuraminidase diffraction data were collected photographically to 3.1 Å resolution using the oscillation method. Crystal alignment and data processing were performed by established techniques²⁸. For the native protein a full data set was collected about the c -axis and 20° of data were collected about each of the [100] and [110] axes. Heavy atom derivatives were prepared by soaking crystals in the usual way. Derivative data were collected only around [100] and [110] due to our failure to grow crystals with suitable morphology reproducibly for c -axis data collection. Table 1 summarizes the data collection statistics. The location of the heavy atom binding sites was determined by difference Patterson and difference Fourier methods. Heavy atom parameter refinement and multiple isomorphous replacement phasing²⁹ resulted in an overall figure of merit of 0.48 for 9,866 reflections to 3.1 Å. Details of the phasing are given in Table 2. None of the derivatives is truly isomorphous beyond about 4 Å. We have also noticed that the crystal lattice is unstable and that partial dehydration of the crystals or soaking in certain heavy atom compounds alters the space group from body centred to primitive. Although the resulting electron density map was uninterpretable, it clearly showed substantial regions of β -sheet, with the familiar right-handed twist. Some 40 cycles of solvent flattening refinement were performed. The new image was still uninterpretable and the value of that refinement remains to be assessed. This electron density distribution was then used to provide partial structure phase information in the analysis of the RI/5⁺ neuraminidase structure.

RI/5⁺/57 neuraminidase: Data were collected photographically by the oscillation method, and were processed by the method of Rossmann^{30,31}. Partially recorded reflections were not included. Oscillation intervals of 2° were used for the native protein and 4° for a single derivative. The resolution limits of the two data sets are 2.9 Å and 4 Å, respectively. Table 3 gives data collection statistics.

A self-rotation function³² of the RI/5⁺ neuraminidase data between 10 and 6 Å showed a peak with height 0.4 times origin peak, corresponding to a noncrystallographic 4-fold axis parallel to [110], a crystallographic diad. This result was confirmed by a cross-rotation function study in which the Tokyo neuraminidase image referred to above was located in the RI/5⁺ neuraminidase crystal with its molecular 4-fold axis parallel to [110]. This rotation function peak was twice the noise level.

Table 1 Tokyo/3/67 neuraminidase data collection

Compound	No. of observations	No. of reflections	R (%)
Native	45,266	10,953	11.9
Diamino dinitro platinum	50,108	10,484	11.9
Potassium platinum tetrachloride	28,079	7,843	11.6
Double platinum derivative	20,170	7,053	10.2
Mercury phenyl glyoxal (A)	25,300	6,228	13.1
Mercury phenyl glyoxal (B)	28,325	8,606	10.3
Samarium chloride	19,521	5,883	11.3
Sodium tungstate	24,329	8,362	11.8
Sialic acid	26,138	9,313	11.4
Lactose	28,401	8,667	11.3

All derivatives were prepared by soaking crystals in heavy atom solutions with standard buffer 0.01 M Tris-HCl pH 7.0; 10 mM (saturated) diamino dinitro platinum, 3 day soak; 3 mM K₂PtCl₄, 1 h soak; double derivative of the two previous platinum compounds; 5 mM (saturated) mercury phenyl glyoxal, 3 day soak; 5 mM (saturated) mercury phenyl glyoxal, 3 h soak; the two mercury phenyl glyoxal preparations showed different reactivities and produced different derivatives with some common sites; 5 mM SmCl₃, 1 h soak; 5 mM sodium tungstate, 6 day soak; 0.5 mM sialic acid, 21 h soak; 200 mM lactose, 4 day soak. $R = \sum(I_i - \bar{I})/\sum I_i$, summed over all measurements. Observations below 3 standard deviations were excluded.

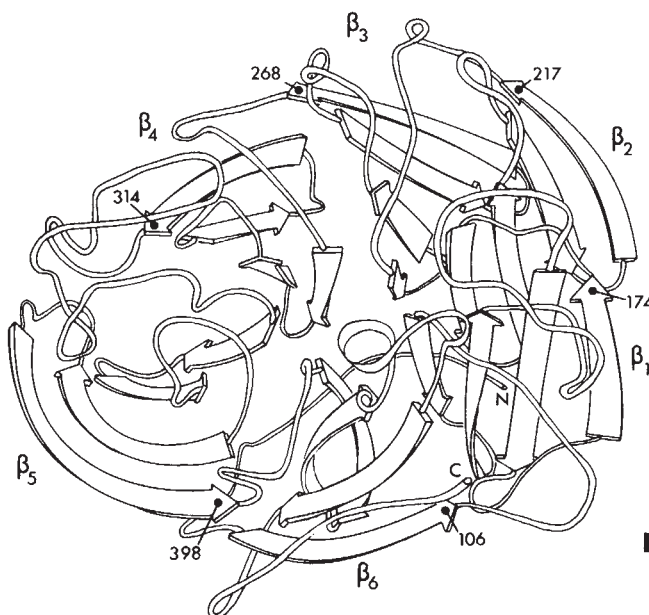


Fig. 1 Diagram of subunit folding of N2 neuraminidase. The molecular 4-fold symmetry axis is indicated and the N-terminal segment emanating from that axis is on the underneath side of the subunit. The view is down the symmetry axis from above. The C terminus is on the outside surface near a subunit interface. The six β -sheets of the propeller fold are near 4 o'clock, 2 o'clock, 12 o'clock, 10 o'clock, 8 o'clock and 6 o'clock, respectively. The N and C termini are indicated as are residue numbers at the C-terminal end of each sheet.

Packing considerations required that the molecular 4-fold axis be coincident with the crystal diad on [110]. An R -factor search for the position of the Tokyo neuraminidase tetramer along this axis yielded a minimum value for R of 0.518 for data between 5 and 4 Å resolution in space group P4₂2₁. A deeper minimum with $R = 0.496$ was found at the same position in space group P4₂2₁, thus permitting a determination of the enantiomorph. Two of the four tetramers in the cell are in the same position in both enantiomorphs.

This trial structure was used to calculate phases for studying the single derivative by difference Fourier methods. The highest four peaks were consistent with the difference Patterson function and were related in pairs around the molecular 4-fold axis.

Fig. 2 Stereo pair of C α skeleton of subunit in the same orientation as Fig. 1. Disulphide bonds are dashed lines, + marks sialic acid binding site²⁴.

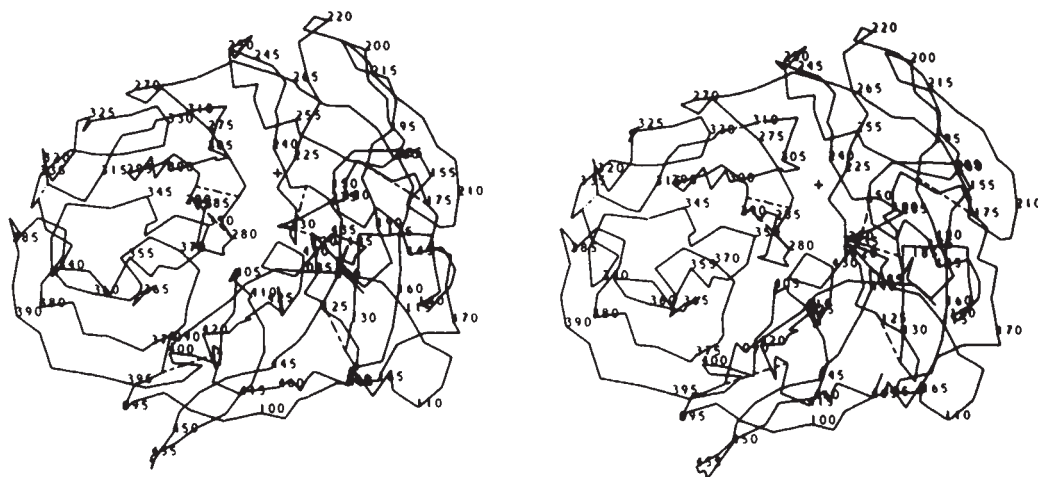


Table 2 Multiple isomorphous replacement phasing of Tokyo/3/67 neuraminidase

Resolution (Å)	11.9	8.5	6.6	5.4	4.5	3.9	3.5	3.1
Diamino dinitro platinum	12.0	11.7	11.8	10.6	10.2	9.6	9.1	8.2
	11.6	6.5	7.3	7.6	7.9	8.9	10.8	14.6
Potassium platinum tetrachloride	11.8	10.5	11.5	10.3	10.3	9.5	9.5	9.0
	12.8	8.8	8.9	9.1	10.4	11.2	12.3	13.1
Mercury phenyl glyoxal (A)	15.5	13.8	13.7	13.7	13.7	12.5	12.0	12.0
	15.5	9.7	9.8	8.9	11.0	12.2	13.9	14.8
Double platinum derivative	16.1	14.2	15.0	13.6	13.8	13.0	12.9	12.2
	8.9	7.2	8.6	8.7	9.1	10.1	11.2	13.6
Samarium chloride	8.4	7.5	7.7	7.3	7.2	6.7	6.7	6.5
	6.3	7.3	9.3	11.4	10.6	11.6	12.2	12.5
Mercury phenyl glyoxal (B)	11.3	10.9	10.6	10.5	10.3	9.9	9.2	9.1
	11.2	7.8	7.6	9.4	10.3	11.9	13.5	13.6
Sodium tungstate	19.4	12.8	9.7	9.2	11.0	11.5	11.3	10.3
	17.2	9.6	9.3	10.0	10.4	11.6	12.0	14.0
Mean figure of merit	0.63	0.68	0.58	0.53	0.53	0.48	0.41	0.35

Numbers for each derivative are in pairs, with r.m.s. heavy atom structure factor above the r.m.s. residual.

The 4 Å single isomorphous replacement phases (Table 4) and the 3.1 Å partial structure phases were combined in the usual way to generate a starting image for noncrystallographic symmetry averaging and refinement^{33,34} over the two independent copies of the molecule in the asymmetric unit. A molecular envelope was drawn on the basis of the multiple isomorphous replacement phased Tokyo neuraminidase image and the single isomorphous replacement phased RI/5⁺ neuraminidase image. Ten cycles of refinement and phase extension out to 2.9 Å resulted in an *R* factor of 0.245, this being a measure of the agreement between observed structure factors and structure factors calculated from the averaged maps on the final cycle. In a further round of refinement this image of RI/5⁺ neuraminidase was rebuilt back into the Tokyo neuraminidase crystal and phases were combined with the multiple isomorphous replacement phases of that structure. This improved Tokyo neuraminidase structure was again rebuilt into the RI/5⁺ neuraminidase crystal and five more cycles of averaging refinement were performed. The final *R* value of 0.246 shows no improvement over that at the previous stage. The high-angle data, however, have improved significantly. The resulting electron density map of RI/5⁺ neuraminidase at 2.9 Å resolution was plotted on transparencies on a scale of 4 Å cm⁻¹ and interpreted in terms of the known sequence^{13,14} and partially known disulphide bonding pattern (C. Ward, personal communication). An electron density map of the Tokyo strain enzyme has also been calculated using phases from the penultimate stage of the refinement process. No significant differences between the two structures are visible at this stage although they differ

at about 20 amino acid residues. We anticipate that a refinement of both structures will eventually indicate the perturbations resulting from the drift in antigenic structure from 1957 to 1967.

Structure description

Low resolution studies have previously established that the tetramer has circular 4-fold symmetry²⁶. The overall dimensions of the neuraminidase head are 100 × 100 × 60 Å. We shall refer to the top and bottom surfaces of the head as being, respectively, the faces outermost and innermost to the viral membrane.

Chain topology: The subunit is folded into an unusual array of six β -sheets. Each sheet has a 'W' topology (+1, +1, +1)³⁵, with four strands connected by reverse turns (Figs 1, 2). This topology is rare in protein structures and its 6-fold occurrence here is noteworthy. When viewed from the top surface of the molecule down the 4-fold molecular symmetry axis, the sheets have the appearance of six blades of a propeller. The first strand of each sheet, near the centre of the subunit, is entered from the top and is nearly parallel to the 4-fold axis. The fourth strand of each sheet is entered from the bottom and is nearly at right-angles to the first strand. Thus, the sheets show the typical right-handed twist³⁶. Not only are the six sheet topologies identical but so too are their connections to each other. The first strand of each sheet is connected across the top of the subunit to the fourth strand of the preceding sheet. The extent to which this topological symmetry extends to the geometrical arrangement of the sheets and their component strands remains to be determined.

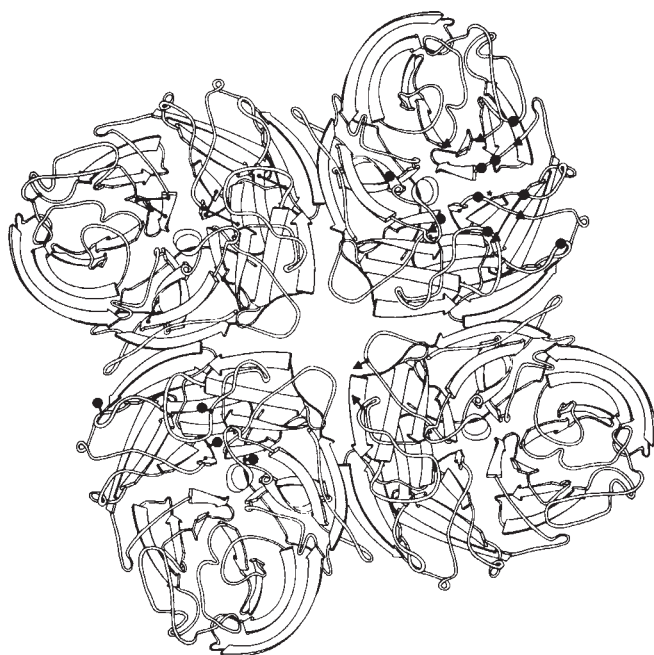


Fig. 3 Diagram of the neuraminidase tetramer viewed from above down the symmetry axis. The four subunits highlight different features of the structure. Top left, disulphide bonds; bottom left, carbohydrate attachment sites at 86, 146, 200, and 234; bottom right, metal ligands Asp 113 and Asp 141; top right, conserved acidic and basic residues in influenza virus A and B neuraminidases. ★ Marks the sialic acid binding site (see accompanying article²⁴).

A convenient notation for describing the location of a residue in the subunit is $\beta_i S_j$ or $\beta_i L_{mn}$ where $i = 1, 6$ for the six β -sheets, $j = 1, 4$ for the four strands per sheet, and where the loop structures between strands are designated L_{01} , L_{12} , L_{23} and L_{34} , being, respectively, the loop connecting a sheet to the preceding sheet in the sequence, and the loops connecting strands 1 and 2, 2 and 3, and 3 and 4 within a sheet. Reading from the N-terminal strand the structure is $\beta_6 S_4$, $\beta_1 L_{01}$, $\beta_1 S_1 \dots \beta_6 L_{23}$, $\beta_6 S_3$, $\beta_6 L_{34}$, C-terminal strand. On all sheets the loops L_{01} and L_{23} are on the top surface of the subunit, and L_{12} and L_{34} are on the bottom surface. The partitioning of the sequence into these structural elements is shown in Table 5.

The N-terminal segment lies across the bottom of the subunit, emanating from a point near the 4-fold axis, and builds the fourth strand of sheet 6 before entering strand 1 of sheet 1. The C-terminal segment is connected to strand 3 of sheet 6 and runs to the top of the molecule at the subunit interface.

It seems most appropriate to characterize the subunit structure as a single domain of six identical folding units. In this way it is more similar to the eight-stranded parallel β barrels of triose phosphate isomerase³⁷ and pyruvate kinase³⁸ than say, to the immunoglobulins³⁹ where the domain structures are self-contained and individually stable.

Disulphide bonds: The disulphide bonding pattern was partially known from chemical studies. The chain folding confirms the connection Cys 318–Cys 337, the predicted connection Cys 175–Cys 193 and one unspecified connection between the N- and C-terminal cyanogen bromide fragments (C. Ward, personal communication). The completed list of disulphide bonds is Cys 92–Cys 417, Cys 124–Cys 129, Cys 175–Cys 193, Cys 183–Cys 230, Cys 232–Cys 237, Cys 278–Cys 291, Cys 280–Cys 289, Cys 318–Cys 337 and Cys 421–Cys 447 (Fig. 3). Chemical studies have now confirmed six of these pairs⁴⁰. This pattern of disulphide bonding reflects the sequential arrangement of β -sheets from N to C termini. The only bridge between linearly distant residues is from near the two termini of the heads (residues 92 and 417) and connects the N-terminal strand to the bottom of sheet 6 at $\beta_6 L_{12}$. It is particularly interesting that the disulphide-rich regions 230–237 and 278–291 are

Table 3 RI/5⁺/57 neuraminidase data collection

Compound	No. of observations	No. of reflections	R(%)
Native	56,863	21,707	12.0
Diamino dinitro platinum	25,838	10,431	11.9

5 mM diamino dinitro platinum in 0.75 M sodium citrate pH 7.0, 2 day soak. $R = \Sigma(I_i - \bar{I})/\Sigma I_i$, summed over all measurements greater than 2 standard deviations.

Table 4 Single isomorphous replacement phasing of RI/5⁺/57 neuraminidase

Resolution (Å)	16.5	9.9	7.1	5.5	4.5	4.0
Diamino dinitro platinum	2.00 0.94	1.81 0.92	1.51 0.87	1.56 0.96	1.58 1.20	1.60 1.56
Mean figure of merit	0.62	0.50	0.45	0.39	0.30	0.19

Numbers are in pairs, with r.m.s. heavy atom structure factor above the r.m.s. residual

Table 5 Assignment of sheet and loop structure in N2 influenza virus neuraminidase

	$i = 1$	2	3	4	5	6
$\beta_1 L_{01}$	107–119	175–177	218–227	269–277	315–350	399–403
$\beta_1 S_1$	120–123	178–185	228–233	278–283	351–356	404–414
$\beta_1 L_{12}$	124–129	186–187	234–236	284–285	357–358	415–416
$\beta_1 S_2$	130–134	188–194	237–242	286–291	359–366	417–428
$\beta_1 L_{23}$	135–156	195–199	243–250	292–295	367–371	429–437
$\beta_1 S_3$	157–162	200–207	251–259	296–304	372–382	438–447
$\beta_1 L_{34}$	163–169	208–209	260–261	305–306	383–386	448–450
$\beta_1 S_4$	170–174	210–217	262–268	307–314	387–398	95–106

The N-terminal strand (84–94) and the C-terminal strand (451–469) are appendages at the bottom of the subunit and at the subunit interface, respectively. Not all of the residues assigned to the strands of the sheet are in regular β conformation.

adjacent to residues implicated in catalytic activity²⁴. These regions contain disulphide bonds between $\beta_3 S_1$ and $\beta_3 S_2$ and between $\beta_4 S_1$ and $\beta_4 S_2$, respectively, and the loops $\beta_3 L_{01}$ and $\beta_4 L_{01}$ contain conserved charged residues²⁴. Disulphide bonds joining adjacent strands of regular β -structure are not strictly allowable⁴¹. Apparently the sheet structure of these regions is perturbed by the interchain covalent linkage.

N1 type neuraminidase contains an additional cysteine at residue 146^{17,18} (corresponding to residue 161 in the N2 type sequence). This residue is possibly disulphide bonded to its counterpart on a neighbouring monomer (see below). The bond between residues 175 and 193 is not found in N1^{15,16} or influenza B²² neuraminidase. It joins the end of β -sheet 1 ($\beta_1 S_4$) with the centre of β -sheet 2 ($\beta_2 S_2$) in the N2 structure. An additional cysteine at a position analogous to 251 in the N2 sequence has been found in influenza B neuraminidase²². This residue is distant from the 4-fold axis and would have to exist as free cysteine if present.

Carbohydrate: Oligosaccharides are attached at asparagine residues 86, 146, 200 and 234¹⁴ as shown in Fig. 3. A fifth potential glycosylation site, Asn 402, carries no carbohydrate in Tokyo neuraminidase⁴². Residues 86 and 234 are on the bottom face of the monomer ~ 11 Å apart. Little, if any, electron density is visible for the sugars in either case. Residue 146 is on the top surface of the molecule in $\beta_1 L_{23}$ and the oligosaccharide attached there is of the complex type and is serologically cross-reactive with chick cell host antigen⁴². This is the only glycosylation site conserved in all known sequences^{13,14,17–22}. It is also unique in containing a significant

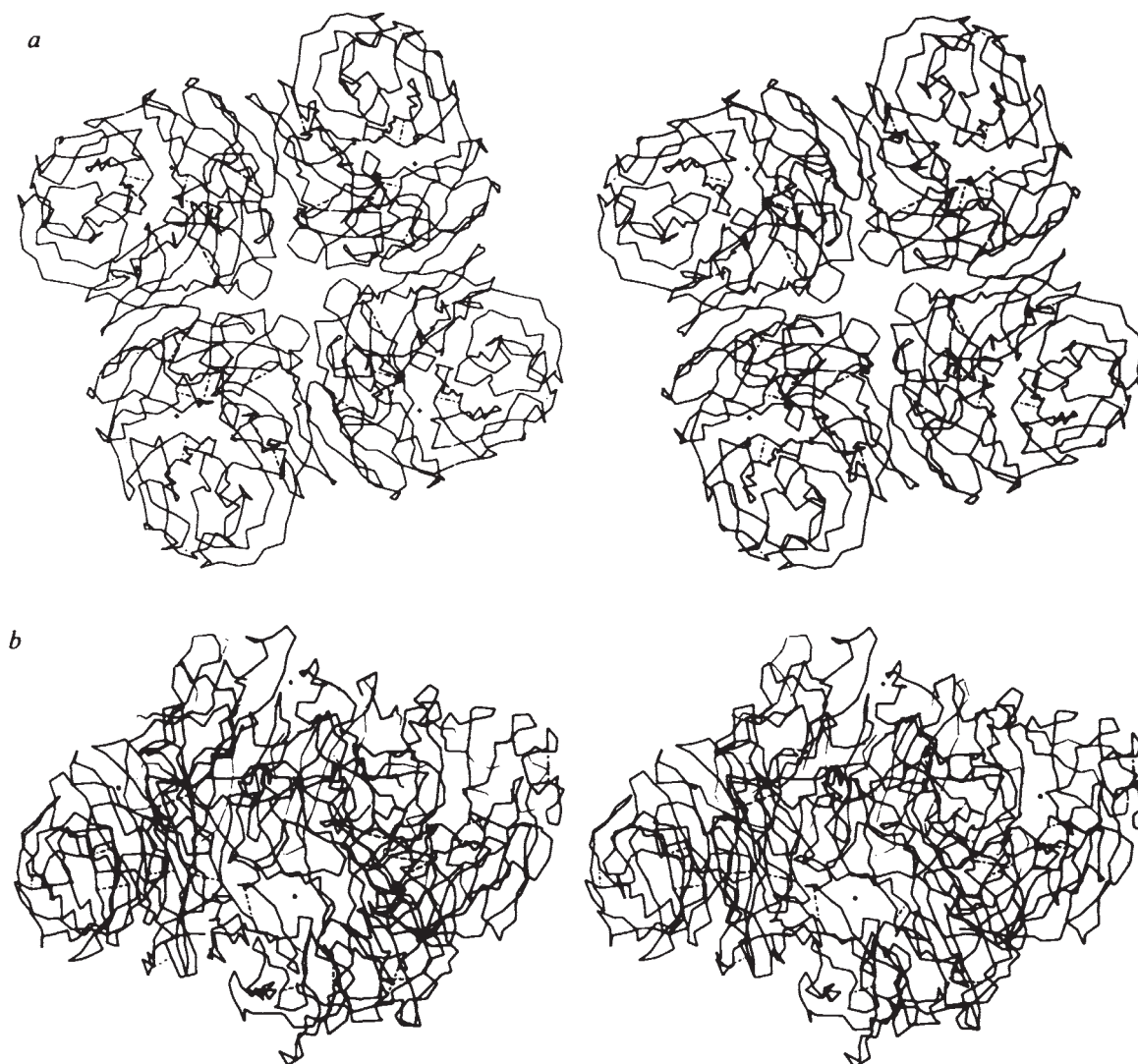


Fig. 4 *a*, Stereo pair of C^α skeleton of the tetramer in the same orientation as Fig. 3. *b*, Stereo pair of C^α skeleton of the tetramer viewed nearly at right angles to the molecular symmetry axis. The active sites of the subunits, marked by a dot, are seen to be oriented towards the four upper corners of the box-shaped neuraminidase head²⁴.

amount of *N*-acetylgalactosamine⁴². The only carbohydrate plainly visible in the present electron density map is that attached at residue 200 on β_2S_3 . This site is near the surface of the tetramer on a subunit interface and the sugar residues seem to interact primarily with surface residues on the neighbouring monomer on β_5S_4 .

We note that the two other glycoproteins whose three-dimensional structures are known also have carbohydrate attached at a subunit interface. In immunoglobulin $\gamma 1$ heavy chains, carbohydrate is attached to the CH2 domain in such a way that the association of the two CH2 domains in the dimeric molecule is different from the association found between other pairs of constant domains⁴³. The haemagglutinin of influenza virus, like the neuraminidase reported here, has a number of glycosylation sites⁴⁴, and one of them occurs at a subunit interface⁴⁵. Approximately half of the carbohydrate associated with the neuraminidase is located in the N-terminal membrane-bound stalk^{42,46} where it might, at least in part, be involved in interchain contacts.

The absence of carbohydrate at residue 402 (β_6L_{01}) in Tokyo neuraminidase is especially interesting. The side-chain conformation in the vicinity of residue 402 is such that Arg 403 is on the surface, rather than buried, and its orientation could prevent glycosylation at Asn 402, provided, of course, that this part of the three-dimensional structure were folded before the glycosyl

transferases were active in this region⁴⁷. There is a significant level of sequence variation in the vicinity of residues 400–403 in field strains, suggesting that they may be part of an antigenic determinant²⁴.

Metal binding sites and subunit interfaces: Calcium ions have been implicated in stabilizing and activating *V. cholerae* neuraminidase⁷. The sensitivity of the influenza protein to dialysis or incubation at elevated temperature is strain-dependent⁴⁸. In the course of preparing derivatives of Tokyo neuraminidase, Sm^{3+} was observed to bind on the molecular 4-fold axis. This binding site is now seen to be in the vicinity of Asp 113 (β_1L_{b1}) and Asp 141 (β_1L_{23}). Given the proximity of these residues to the 4-fold axis (Figs 3, 4), there are eight acid groups in this cluster in the tetramer and it is likely that more than one calcium ion is bound between them. The situation may be similar to that in thermolysin⁴⁹ where a single lanthanide ion replaces two calcium ions at the interface of the two structural domains of that enzyme.

The only other intimate interaction around the 4-fold axis is in the vicinity of His 168, (β_1L_{34}). Note that His 168, near the 4-fold axis in N2, has no counterpart in N1. Nearby, at a position analogous to Asn 161 in N2 sequences, N1 structures have Cys 146^{17,18}. Equivalent C^α atoms on neighbouring subunits are ~ 15 Å apart. Intersubunit disulphide bonding through this residue in N1 is therefore only possible with a significant

rearrangement of the end of β_1S_3 and the following loop β_1L_{34} . No cysteine in the stalk region of N1 structures is likely to be close enough to bridge to Cys 146.

The subunit interface is extensive and includes both hydrophobic and salt-link interactions. The outside strands of sheets 1 and 6 (β_1S_4 and β_6S_4) on neighbouring subunits are sufficiently close together to characterize their interaction as a continuation of the β -sheet across the interface (Figs 3, 4). Details must await model refinement, but only one or two peptide groups on each strand are close enough to contribute to this interaction. The extension of β -sheet hydrogen bonding across a subunit interface is not uncommon but in other known examples to date it has always involved equivalent strands related by a 2-fold symmetry axis⁴¹. In the case of neuraminidase the sheet twists through 180° in passing from the centre of one subunit to the centre of the next.

The N-terminal region: Two different cleavage sites for pronase in Tokyo neuraminidase have been positively identified at residues 74 and 77¹⁴. A third site on the C-terminal side of Cys 78 can be inferred from the observation that the subunits in the NA heads are sometimes disulphide bonded in pairs and sometimes not. Cys 78 presumably mediates this covalent dimerization. Electron density for Tyr 84 is clearly visible in the present map 19 Å from the 4-fold axis on the underside of the subunit. Beyond this point, the density falls to background levels. A tentative assignment of the chain through weak density from Tyr 84 back to Pro 79 in strong density has been made but this chain segment is not included in any of the present figures. Such a structure would give rise to a hairpin loop in intact neuraminidase, with Cys 78 at the top of the loop, and at the top of a column of electron density seen on and around the 4-fold axis in a 5 Å resolution map of Tokyo neuraminidase²⁶. This feature is not prominent in the high resolution image. A number of factors may be responsible, including heterogeneity of the pronase cleavage, sequence variability between RI/5⁺ and Tokyo neuraminidase, and failure of this segment strictly to observe the 4-fold symmetry axis.

The N-terminal 73 residues of the intact N2 structure fall into three regions⁵⁰. Residues 1–6, conserved in N1 and N2, are on the cytoplasmic side of the membrane. The hydrophobic sequence from residues 7–35 spans the lipid bilayer and the heavily glycosylated region at 36–73 completes the stalk structure to which the heads are attached. Electron microscope evidence^{1,51} suggests that the stalk is thin. The four chains in the tetramer will be constrained to lie close together at least at places where intersubunit bonding occurs. Neuraminidase from B/Lee has only a single cysteine in the stalk at residue

54, and SDS-released enzyme of this strain is composed of two dimers⁵². It is therefore likely that strains with a number of cysteine residues in the stalk will exist as a population of covalently linked dimers and tetramers depending on the numbers of possible cross-links involved.

Structures of other neuraminidases: A comparison of sequences from influenza A (N1^{17,18} and N2^{13,14,19–21} subtypes) and B²² shows conserved residues with crucial structural and functional roles. Many of the half cysteines can be aligned in all sequences and catalytic charged residues are conserved²⁴. The cross-reactive carbohydrate attachment site, at residue 146⁴² in the N2 sequence, is conserved. A number of glycine residues (164, 186, 244, 348) found in tight bends of the N2 structure are also conserved. An ion pair Glu 375–Arg 394 in N1 and N2 becomes Arg 375–Asp 394 in the B sequence. In addition, conserved hydrophobic residues are found at the various sheet interfaces. It seems, therefore, most probable that all influenza virus neuraminidases show the propeller framework topology described here for the N2 structure.

No primary structure information is available for comparison with bacterial or mammalian neuraminidases. Some of their physical characteristics are similar to those of influenza virus neuraminidase. It has been suggested that human leukocyte neuraminidase is a tetramer of four identical subunits with molecular weights similar to that found for placental neuraminidase⁵³ and the influenza enzyme, that is, 63,000. Mammalian neuraminidases are most commonly associated with membranes, for example, in brain, liver, heart, leukocytes and spermatozoal acrosomes⁵³. Soluble forms are also known to occur in liver.

Conclusions

Influenza virus neuraminidase provides the first known example of a tetrameric protein with circular 4-fold symmetry. The polypeptide chain folds into six, topologically identical, four-stranded, antiparallel β -sheets which are themselves arranged like the blades of a propeller. Calcium ions are believed to be bound on the 4-fold axis between a cluster of eight acidic groups. The most C-terminal of the five glycosylation sequences carries no carbohydrate.

The location of the enzyme active centre and its relationship to variable regions, implicated in binding antibody, are discussed in the accompanying article²⁴.

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Structure of the catalytic and antigenic sites in influenza virus neuraminidase

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The catalytic sites of influenza virus neuraminidase are located on the upper corners of the box-shaped tetramer that forms the head of the molecule. Antigenic determinants form a nearly-continuous surface across the top of the monomer encircling the catalytic site. Approximately the same number of amino acid sequence changes occurred in these determinants between the years 1968 and 1975 as occurred in the antigenic sites of influenza virus haemagglutinin in the same period.

UNLIKE most other viral antigens, those of influenza continue to show structural variation in response to the challenge of an immune host population. However, this structural variation may not include amino acids required for special function such as those in the catalytic centre of the neuraminidase or in the receptor binding pocket of the haemagglutinin. Apparently, functional amino acids are in privileged locations in the viral protein structure where they are either (1) inaccessible to antibodies, (2) accessible to antibodies but only in such a way that non-essential amino acids also form part of the antigen-antibody complex, or (3) simply not antigenic.

We have determined the three-dimensional structure of influenza virus neuraminidase (see accompanying article¹) and discuss here three aspects of that structure: the catalytic site, the location of antigenic determinants and the relative structural arrangements of these two biological functions. The notation for identifying strands and loops on the six β -sheets in the structure is given in the accompanying article¹.

The enzyme active site

Neuraminidase EC 3.2.1.18 shows mild product inhibition². Sialic acid binds to the enzyme with an affinity constant of approximately 1 mM. A three-dimensional data set of X-ray diffraction data to 3 Å resolution has been collected from Tokyo neuraminidase crystals soaked in 0.5 mM sialic acid¹. A Fourier map of the differences between this derivative and the native enzyme shows a prominent feature in a large pocket on the top surface of the subunit. At 5 Å resolution, this peak is 2.1 times the largest noise peak and at 3.5 Å resolution it is equal in height to the same noise peak at one of the heavy atom derivative phasing sites. The pocket enclosing the sialic acid binding site is rimmed by 12 loops of chain, 6 being the loops connecting strand 4 of one sheet with strand 1 of the following sheet (L_{01}), and 6 being loops between strands 2 and 3 within a sheet (L_{23}). There is an unusually large number of charged residues in the pocket and around its rim. These charged amino acids are conserved in all A and B strain influenza virus neuraminidase sequences known to date³⁻¹⁰. They are Arg 118, Glu 119 (on β_1L_{01}), Asp 151, Arg 152 (β_1L_{23}), Asp 198 (β_2L_{23}), Arg 224, Glu 227 (β_3L_{01}), Asp 243 (β_3L_{23}), His 274, Glu 276, Glu 277 (β_4L_{01}), Arg 292 (β_1L_{23}), Asp 330, Lys 350 (β_5L_{01}), and Glu 425 (β_6S_2). Other conserved residues found close to the sialic acid binding site include Tyr 121 (Phe in N1^{5,6} and B¹⁰ strains), Trp 178 and Leu 134. Similar hydrophobic residues are found in the sialic acid binding pocket of wheat germ agglutinin¹¹ and the putative pocket of influenza virus haemagglutinin¹². Superficially, the main distinguishing feature of the enzyme active site against the specificity pocket of the agglutinins is its large size and the charge clustering. These conserved residues

around the sialic acid binding site are shown in a diagram of the subunit in Fig. 1. Details of the orientation of the sialic acid in the binding pocket and its interaction with the protein must await model refinement.

Three-dimensional X-ray diffraction data from a derivative of Tokyo neuraminidase soaked in 0.5 M lactose have been collected and processed¹. A difference Fourier map with the native enzyme data set shows no significant features. This suggests that Tokyo neuraminidase shows little, if any, subsite specificity for lactose adjacent to sialic acid. The enzyme specificity is believed to be directed at the configuration of the linkage rather than to an adjacent saccharide unit bound in a subsite².

The tetrameric neuraminidase is reported to show multiphasic kinetics¹³. The distance between the active centres across the subunit interface is 42 Å (Figs 3 and 4 of the accompanying article¹).

Antigenic variation

Role of antibodies to influenza virus neuraminidase: Antibodies to neuraminidase do not neutralize virus infectivity¹⁴ (except at very high concentrations), this is consistent with data that

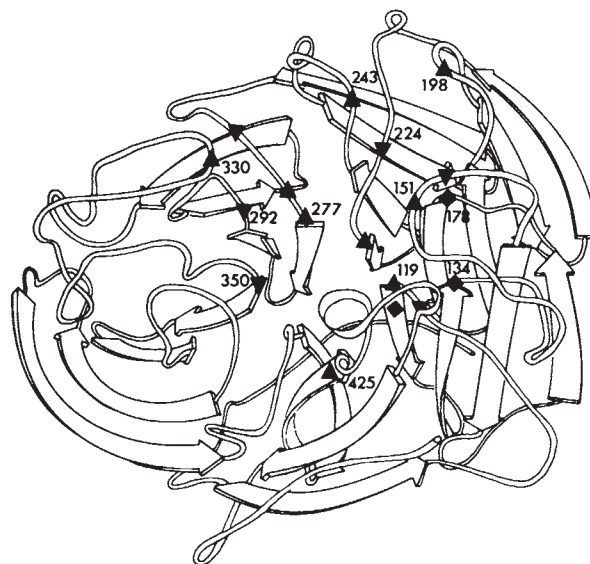


Fig. 1 Conserved residues near the sialic acid binding site. ▲, Glu 119, Asp 151, Asp 198, Glu 227, Asp 243, Glu 276, Glu 277, Asp 330, Glu 425. ▼, Arg 118, Arg 152, Arg 224, His 274, Arg 292, Lys 350. ◆, Tyr 121, Leu 134, Trp 178.

Table 1 Amino acid sequence changes in N2 neuraminidase, indicating the location in the three-dimensional structure

	RI/5 ⁻ /57	RI/5 ⁺ /57	Tokyo/3/67	Aichi/2/68	NT/60/68	Eng/42/72	Udorn/ 307/72	Vic/3/75	
86	Asn	Asn	Asn	Asn	Asn	Lys	Asn	Asn	N-terminal strand, loss of glycosylation site in Eng
93	Gln	Gln	Gln	Gln	Gln	Lys	Lys	Lys	N-terminal strand, bottom surface residue
126	Pro	Pro	Pro	—	His	Pro	Pro	Pro	β_1 L ₁₂ , bottom surface residues
127	Gly	Gly	Val	—	Gly	Gly	Gly	Arg	
141	Asp	Asp	Asp	Asp	Asp	—	Asp	Glu	β_1 L ₂₃ , acid group probably involved in binding metal ion on the 4-fold axis
147	Gly	—	Asp	Asp	Asp	—	Asp	Asp	β_1 L ₂₃ , side-chain orientations uncertain, probably external but could be covered by carbohydrate at Asn 146
149	Ile	—	Val	Ile	Ile	—	Ile	Ile	
153	Ile	Ile	Ile	Ile	Ile	Ile	Thr	Thr	β_1 L ₂₃ , external, accessible to Ig
172	Lys	Lys	Arg	Arg	Arg	Arg	Arg	Arg	β_1 S ₄ , conserved charge at subunit interface, near Asp 103 on a neighbouring subunit
176	Val	Val	Ile	—	Ile	—	Ile	Ile	β_2 L ₀₁ not surface accessible
177	Ala	Ala	Ala	—	Ala	—	Gly	Ala	
194	Val	—	Ile	—	Ile	—	Val	Val	β_2 S ₂ buried
197	Asp	—	Asp	—	Asp	—	Tyr	Tyr	β_2 L ₂₃ surface residues accessible to Ig
199	Arg	—	Asp	—	Lys	—	Lys	Lys	
221*	Asn	Asn	Asn*	Asn	Asn	Asn	Asn	Asn	β_3 L ₀₁ surface residue accessible to Ig, Asn → His in monoclonal variant of Tokyo
253	Arg	Arg	Arg	Arg	Arg	Arg	Lys	Lys	β_3 S ₃ conserved charge, side chain orientation unclear probably in surface
258	Lys	Lys	Glu	Glu	Glu	Glu	Glu	Glu	β_3 S ₃ bottom surface residue
269	Ser	Ser	Ala	Ser	Ala	Ser	Ser	Ser	β_4 L ₀₁ surface residue, accessible to Ig, but not close to any other site of variation
275	Ile	Val	Val	Val	Val	Val	Val	Val	β_4 L ₀₁ buried
286	Asp	Asp	Gly	Gly	Gly	Gly	Gly	Gly	β_4 S ₂ bottom surface residue
303	Ile	Val	Val	Val	Val	Val	Val	Val	β_4 S ₃ buried
307	Met	Met	Met	Met	Met	Val	Val	Val	
308	Glu	Glu	Glu	Glu	Glu	Lys	Lys	Lys	β_4 L ₃₄ bottom surface residue
328	Asn	—	Asn	Asn	Asn	—	Asn	Lys	
329	Asp	—	Asp	Asp	Asp	—	Asn	Asn	β_5 L ₀₁ highly convoluted loop separated into two by cysteine 337. Accessible to antibody
331	Ser	—	Arg	Arg	Arg	—	Arg	Arg	
334	Asn	—	Asn	—	Asn	—	Asn	Ser	
336	Asn	—	Asn	—	Asn	—	Tyr	Tyr	
339	Asp	—	Asn	—	Asn	Asn	Asn	Asn	β_5 L ₀₁ conserved charge in field strains but Arg → Ile in monoclonal variant of Tokyo
344	Arg	—	Arg*	—	Arg	Arg	Lys	Lys	
346	Asn	Asn	Thr	Asn	Asn	Asn	Asn	Ile	β_5 L ₀₁ surface residue, accessible to Ig
347	Pro	Pro	Gln	Gln	Gln	His	His	His	β_5 L ₀₁ buried. Presumably this loop structure alters significantly with some substitutions
356	Asn	Asx	Asn	Asx	Asn	Asx	Asp	Asp	β_5 S ₁ bottom surface
358	Asp	Asx	Asn	Asx	Asp	Asx	Asn	Asn	β_5 L ₁₂ bottom surface
360	Val	Leu	Leu	Val	Val	Val	Val	Val	β_5 S ₂ buried
367	Asn	Asn	Ser	Ser	Ser	Ser	Ser	Ser	β_5 L ₂₃ surface residues accessible to Ig, Lys → Glu in monoclonal variant of Tokyo
368	Lys	Lys	Lys*	Lys	Lys	Lys	Glu	Glu	
369	Glu	—	Asp	Asp	Asp	Asp	Asp	Asp	β_5 S ₄ surface residues partly covered by carbohydrate on Asn 200 of neighbouring subunit
370	Ser	—	Leu	Leu	Leu	Ser	Ser	Ser	
390	Ser	Ser	Ser	Ser	Ser	Ser	Leu	Leu	β_6 L ₀₁ surface residues accessible to Ig
392	Val	Val	Ile	Ile	Ile	Ile	Ile	Ile	
400	Asn	Ser	Ser	Ser	Ser	Ser	Ser	Ser	β_6 L ₂₃ in turn of α -helix, accessible to Ig
401	Asn	Asp	Asp	Asp	Asp	Asp	Asp	Ala	
403	Trp	Arg	Arg	Arg	Arg	Arg	Arg	Arg	C-terminal strand in surface, but probably not accessible to Ig
431	Pro	Pro	Lys	Lys	Lys	Glu	Glu	Glu	
434	Thr	Thr	Thr	Thr	Ala	Thr	Thr	Thr	C-terminal strand buried
463	Asn	—	Asn	—	Asn	—	Asp	Asp	
466	Phe	—	Phe	—	Phe	—	Leu	Leu	

—, Not determined.

* Residues of Tokyo/3/67 observed to change in monoclonal variants.

neuraminidase is not required by the virus to infect cells. Anti-neuraminidase antibodies do, however, modify the disease, and they reduce both the pulmonary virus titre and the extent of lung lesions¹⁵. It seems likely, therefore, that antibodies to the neuraminidase have some role in the epidemiology of influenza. **Amino acid sequence changes in the neuraminidase of field strains:** The complete sequences of five N2 neuraminidases are now known from the following isolates: RI/5⁻/57³, Tokyo/3/67⁴, NT/60/68⁸, Udorn/307/72⁷ and Vic/3/75⁹. In addition, partial sequence data from peptide mapping are available for RI/5⁺/57, Aichi/2/68 and Eng/42/72¹⁶. Table 1 lists all the sequence changes observed in the neuraminidase heads of these eight variants and indicates the position of the changed residue in the three-dimensional structure.

Sequence changes in the neuraminidase of 'monoclonal variants': Antigenic variants of neuraminidase have been selected with a frequency of $\sim 10^{-5}$ after a single passage of A/Tokyo/3/67 influenza virus in the presence of monoclonal

antibody to the neuraminidase¹⁷. These 'monoclonal variants', like those of influenza virus haemagglutinin previously described¹⁸, do not bind at all to the antibody used for their selection, and each shows a single change in the amino acid sequence of the neuraminidase.

Three different variants of neuraminidase, grown from Tokyo/3/67, have been partially sequenced, and they show sequence changes at residues 221, 344 and 368¹⁶. The latter two changes occurred in chain segments also showing high variation in field strain sequences. The other change, at residue 221, is in a segment of structure undergoing no change in the field strains. However, the three-dimensional structure shows residue 221 to be on an adjacent loop to 197–199, where variation is found in field strains.

Antigenic determinants: The sequence changes observed in the monoclonal variants of neuraminidase probably occur in antigenic determinants as, by implication, do the corresponding changes in the field strains. Other sequence changes in the field

Table 2 Shortest distances (Å) between C α atoms of the seven variable segments in the three-dimensional structure

Family Chain	I	II	III	IV	V	VI	VII
segments 328-336 339-347 367-370 400-403 431-434 197-199 153							
328-336	—	6	11	20	19	>	>
339-347	6	—	6	15	16	>	>
367-370	11	6	—	9	14	>	>
400-403	20	15	9	—	12	(16)	(17)
431-434	19	16	14	12	—	>	>
197-199	>	>	>	(16)	>	—	—
153	>	>	>	(17)	>	8	—
NANA	10	10	10	13	11	10	13

>, Distances greater than 20 Å. Numbers in parentheses indicate distances measured across the subunit interface. Also shown are the closest distances from the observed binding site of sialic acid (NANA) on the enzyme. Roman numerals refer to the family of the antigenic determinant defined as the association of a particular chain segment in the binding pocket of an antibody.

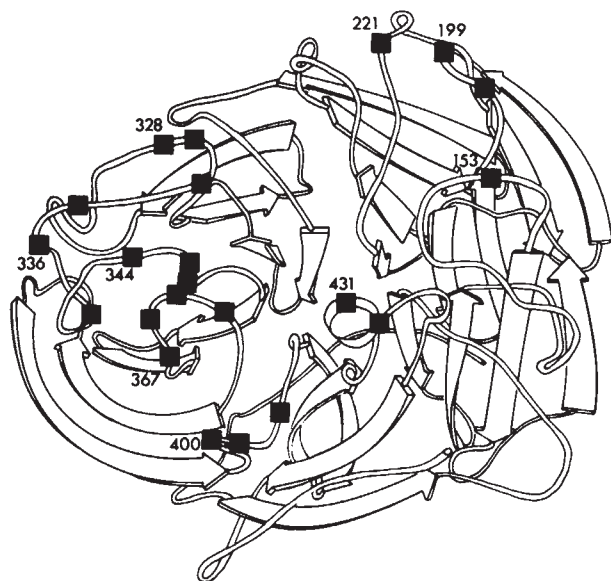


Fig. 2 Diagram of the neuraminidase subunit structure flagging residues in the seven variable segments referred to in the text: 153, 197, 199, 221, 328, 329, 331, 334, 336, 339, 344, 346, 347, 367-370, 400, 401, 403, 431, 434.

strains, however, may or may not be linked to binding sites for antibodies. On the basis of their accessibility to antibodies and the frequency of amino acid sequence changes in their vicinity, seven chain segments can be identified as potential contributors to interactions with antibody. They are residues 153, 197-199, 328-336, 339-347, 367-370, 400-403 and 431-434. Variable residues in these segments are flagged on a diagram of the subunit in Fig. 2. The loop β_5L_{01} , which carries 328-347 is highly convoluted and separated into two loops by the cysteine at residue 337.

The question of how these seven segments are grouped into antigenic determinants is best approached by considering the size and shape of that portion of the surface of antibodies which associates with antigen. The six hypervariable loops of antibodies typically form a pocket at the distal end of the Fab arms of the immunoglobulin molecule¹⁹⁻²¹. The size of this pocket is highly variable. The size of the surface which the hypervariable loops present is less variable and measures ~30 Å in diameter. Thus, amino acids binding in the pocket of the antibody might form part of a single antigenic determinant with other residues on the antigen 15 Å distant. These residues would associate with the periphery of the variable surface on the antibody. Table 2 shows the shortest distance between C α atoms of all segments to all other segments and reveals two

groupings of segments: one grouping is defined by 153 and 197-199, and the second by the remaining segments.

Seven families of determinants are envisaged. Each family is characterized by an association of one of the seven segments with the pocket of the antibody; they are designated I to VII as shown in Table 2. Families I-V show no overlap (at the 15 Å cut off) with families VI and VII. Inclusion of the segment 400-403 on the neighbouring monomer in the family VI and VII determinants would see all determinants linked to all others. At the 15 Å level, families I-V all overlap, although there may well be members of families I and V which do not involve the loop 367-370. We emphasize here that the size of any determinant can only be crudely estimated and the use of a 15 Å radius is no more than a guideline. It appears that, at most, three antibodies could bind to each neuraminidase subunit recognizing family I, V and VI or VII determinants. Antibodies recognizing family II, III or IV determinants will reduce this number to two and possibly even only one depending on the extent of family VI or VII determinants across the subunit interface.

A comparison of the sequences of NT/60/68⁸, Udorn/307/72⁷ and Vic/3/75⁹ allows a determination of the frequency of amino acid changes in regions of the neuraminidase believed to be important in binding antibodies (Fig. 2). Ten such changes occurred between 1968 and 1972 and a further five between 1972 and 1975. In the haemagglutinin over the same 7-yr period, eight and six changes, respectively, occurred in the antigenic sites²².

Antigenic determinants and enzyme active site

Figure 3 gives a stereo view of the active site and antigenic determinants. The shortest distances from all of the variable segments to the observed site of binding of sialic acid in the crystals is displayed in Table 2. All segments are sufficiently close to inhibit activity with macromolecular substrates. The effect of different monoclonal antibodies on the enzyme activity varies widely and depends on both the antibody and the substrate used. With fetuin as substrate, some antibodies which bind to neuraminidase inhibit enzyme activity while others do not^{17,23}. In some cases enhancement of neuraminidase activity has been reported¹³. Other experiments have shown that activity towards *N*-acetyl neuraminyl lactose was not always inhibited by antibody²⁴. One such antibody, S10/1, sees residue 368 as part of the determinant, although whether in the pocket or in the periphery of the antibody is not known. The Fab fragment of that antibody has been crystallized and its three-dimensional structure is being determined²⁵. Three different monoclonal antibodies whose binding to neuraminidase is affected by changes at residue 344 all inhibit activity towards *N*-acetyl neuraminyl lactose²⁴.

In some cases the variable segments contain residues which are conserved in all known influenza virus neuraminidase sequences (N1^{5,6}, N2^{3,4,7-9}, B/Lee¹⁰) and have been implicated in the catalytic activity. Asp 151 and Arg 152 are adjacent to the variable residue 153. Asp 198 is between sites of variation at 197 and 199 and Asp 330 is part of the convoluted loop β_5L_{01} . In all these cases the side-chain orientation of the conserved residue is towards the sialic acid binding site pocket whereas the variable residues are oriented outwards in the surface.

Active sites of protein molecules are usually formed from concave surfaces. This feature alone offers some protection to functionally important residues of viral proteins from host antibodies. Careful model building experiments will be required to establish whether or not any of the conserved catalytic residues of neuraminidase are accessible to antibody. In the event that such residues participate in binding to antibodies, they must strictly be considered part of the antigenic structure of the protein. In the case of neuraminidase, however, it is clear that if an antigenic determinant were to embrace some of the catalytic residues, a significant part of the determinant would

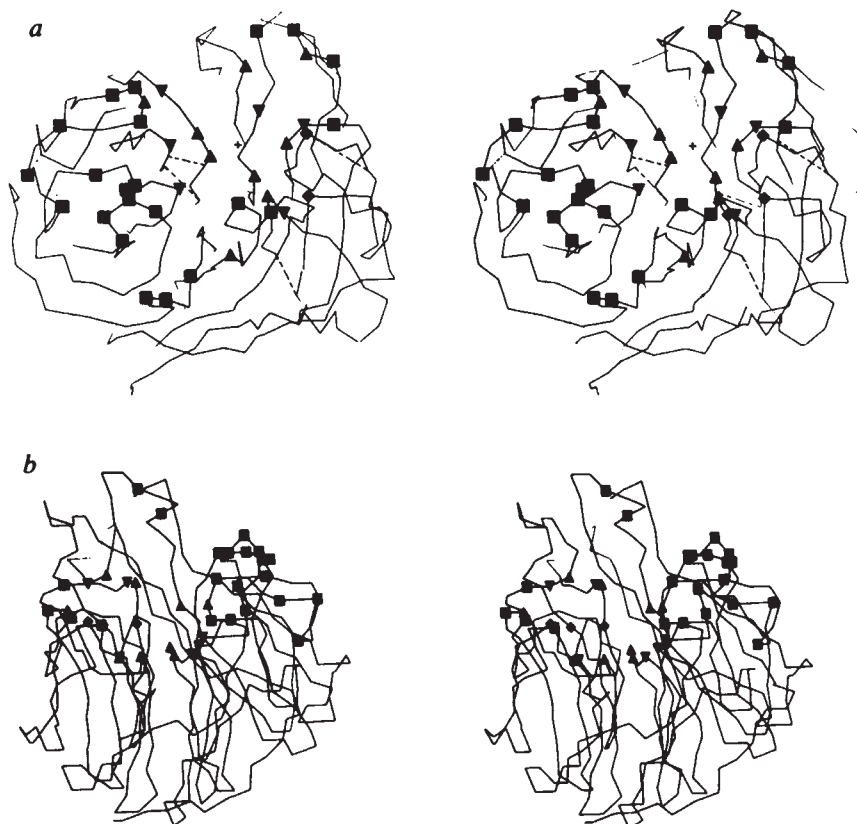


Fig. 3 Stereo pair showing composite of catalytic site and antigenic determinants. Residue flags as in Figs 1 and 2. *a*, View from above, normal to the viral membrane, as in Figs 1 and 2. The cross marks the sialic acid binding site. *b*, View from the side, parallel to the viral membrane, with the 4-fold axis of the tetramer at left rear.

comprise the variable surface loops showing field strain variation.

Conclusions

Influenza virus neuraminidase has a box-shaped globular head attached to a thin stalk by means of which it is embedded in the viral membrane. The four catalytic sites of the tetrameric head are on that surface of the head distal to the membrane.

The product of catalysis, sialic acid, is observed to bind in a large pocket on the distal surface, flanked and surrounded by nine acidic residues, six basic residues and three hydrophobic residues, all of which are strictly conserved in all known influenza virus neuraminidase sequences.

Amino acids which change during antigenic drift are observed to cluster preferentially into the distal surface loops which connect the various strands of β -sheets. Some of these variable residues are immediately adjacent to amino acids implicated in enzyme activity. The variable loops do not segregate into spatially distinct non-overlapping areas of variation. Rather, they form a nearly continuous surface across the top of the subunit, encircling the catalytic site. It remains to be seen whether all of these variable loops can participate in forming

complexes with immunoglobulins. Point mutations in laboratory mutants of neuraminidase selected with monoclonal antibodies indicate that residues 221, 344 and 368 may all form part of antigenic determinants and it is likely that when more mutants are characterized, remaining regions of frequent field strain variation will also be seen to form sites for interaction with antibodies. Between 1968 and 1975 the neuraminidase shows the same number of amino acid sequence changes in the putative antigenic determinants as does the haemagglutinin.

The proximity of the antigenic determinants to the catalytic site suggests that antibodies cannot exert pressure on that site for its modification. Furthermore, it would seem that there are not good prospects for making effective antisera against functionally essential amino acids of the neuraminidase.

The sequence studies and X-ray diffraction studies on the neuraminidase and haemagglutinin²², together with the information on the distribution of antigenic sites delineated with monoclonal antibodies, provide the basis for describing the complete antigenic structure of this animal virus.

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A measurement of the IR flux from a radio lobe

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Prompted by the detection of optical radiation from the extended lobes and jets of some radio galaxies¹⁻³, we have begun a search for IR radiation from such sources. Initially we have observed the western lobe of 3C247 (or 3C247W) at 2.2 μm and measured a flux density of $250 \pm 27 \mu\text{Jy}$ ($m_K = 16.0 \pm 0.1$). This result shows there to be a break in the spectrum between radio and optical wavelengths, such that the IR-optical spectrum is steeper than would be predicted from the radio and optical data alone. If the spectrum of 3C247W is applicable to other sources, then IR searches are a good way of finding high frequency counterparts of lobes of radio galaxies.

Radio galaxies commonly emit the bulk of their radio energy from large extended lobes which are typically several hundred kiloparsecs away from the optical galaxy⁴. The radio spectrum of these lobes is usually a power law with a spectral index of $\alpha = -0.8$, where the flux density $f_\nu \propto \nu^\alpha$. The radio emission is due to synchrotron radiation of relativistic electrons which must have energies of at least $mc^2(mc\nu_{\text{max}}/eB)^{1/2}$ where m is the mass of an electron, c is the velocity of light, e is the charge of a proton, B is the magnetic field strength and ν_{max} is the maximum frequency at which the spectrum is observed to be a power law⁵. If the synchrotron power law spectrum extends as far as 2.2 μm then there must be radiating electrons in the lobe with energies a hundred times greater than can be inferred from the highest frequency radio observations (at 15 GHz). This is the most likely explanation of the IR-optical flux of 3C247W.

We selected the brightest optical identification of a radio lobe which has been reported, as a test case to search for the corresponding IR radiation. This source is 3C247 (reported by Laing *et al.*⁶), which is a 15 arc s double radio source⁷ with some optical emission associated with the western lobe. On the night of 20–21 March 1982, we observed 3C247W with UK IR telescope (UKIRT) sited on Mauna Kea, Hawaii. We used the UKT6 photometer⁸ with the K filter and an aperture of 7 arc s FWHM. An aperture as large as 7 arc s had to be used because the telescope was being buffeted by a strong wind, but this aperture is small enough to ensure that there is no contamination from nearby objects. In a 15-min (real time) integration we measured the 2.2- μm flux density of the lobe to be $250 \pm 27 \mu\text{Jy}$, which is a K magnitude of 16.0 ± 0.1 . The correction for galactic absorption is <0.02 mag as the neutral hydrogen column density is low in this region of the sky⁹. The magnitude scale was calibrated by observing standard stars and the magnitude to flux conversion was performed using the IR flux of α Lyrae as measured by Strecker *et al.*¹⁰.

Laing *et al.*⁶ reported that the optical flux in 3C247W had a magnitude of “ ~ 20 ”, with no specification of probable error. Where necessary, we have arbitrarily assigned a factor of 2 uncertainty (0.75 mag) to the optical flux although the flux is more likely to be on the low side than high since the object does not appear on the red Palomar Sky Survey plate (plate limit, $m = 20$). Combining the optical data, measured at 6,500 $\text{\AA}$, with our own data gives an $R-K$ colour of ~ 4 . If the IR-optical object is actually a star which coincidentally lies near the radio lobe, then it is a very red star, probably of spectral type M7, and certainly later than M4 (ref. 11). Such stars have $V-R \approx 2$, so its V magnitude would be 22. Such stars are predominantly disk stars¹². The surface density of

$m_v = 22$ stars at a galactic latitude of 60° is 1.6×10^3 per square degree per magnitude¹³, therefore the surface density of stars having $m_v = 22 \pm 0.75$ near 3C247 is $\sigma = 1.6 \times 10^3 \times 2 \times 0.75 = 2.4 \times 10^3$ stars per square degree, and the probability of finding a star within 2 arc s of 3C247W by chance is 2.3×10^{-3} . A measurement of the star density on the red Palomar Sky Survey plate in the region of 3C247 indicates that the local star density is lower than the average figure quoted above. In view of this, and the fact that not all $m_R = 20$ stars are M stars, the probability of 2.3×10^{-3} of a chance superposition should be considered an upper limit. The optical object is actually only 1 arc s away from the peak of the radio emission, but *a priori* one might have decided that any object within 2 arc s of the radio peak was possibly associated with the radio source. Laing *et al.*⁶ suggested that the optical object might be a quasar, but again the surface density of quasars with $m_B = 21$ (corresponding to $m_R = 20$) is ~ 150 per square degree^{14,15}, so the probability of finding a quasar within 2 arc s of the radio peak is 1.4×10^{-4} .

Statistically then, it is very likely that the IR-optical source is physically associated with the radio lobe, and a reasonable interpretation of the IR-optical radiation is that it is simply the high frequency extension of the radio synchrotron emission. The spectrum of the lobe is plotted in Fig. 1 using data from the 5-km map of Jenkins *et al.*⁷ and the radio source catalogue of Kuhr *et al.*¹⁶. At 5 GHz two-thirds of the total flux comes from the western lobe, and in plotting Fig. 1 we have assumed that this is true at all radio frequencies. Comparison with the spectrum of the total flux of 3C247 (refs 17, 18) indicates that this should be valid for frequencies above 100 MHz. The immediate conclusion from Fig. 1 is that there is a strong break in the spectral index between the radio and optical regions of the spectrum. At radio wavelengths the spectrum bends downwards slightly, so it would be very useful to have any observations of 3C247W in the range 100 GHz–10 μm to see whether there is a fairly smooth joining of the radio to the IR, or whether there is a large peak in the far IR as a simple extrapolation of the optical-IR spectrum to longer wavelengths would indicate.

There is no spectroscopic redshift for 3C247, but from the magnitude of the central galaxy Laing *et al.*¹⁹ have estimated its redshift to be 0.75. Using this we can calculate some physical parameters for 3C247W. Taking the spectrum to vary as $\nu^{-0.8}$ for frequencies below 1.4×10^{14} Hz and as $\nu^{-1.7}$ above this, the total power output of the lobe is 1.0×10^{46} erg s⁻¹ (taking $H_0 = 50$ km s⁻¹ Mpc⁻¹). The lobe is not resolved on the 5-km map so it must be smaller than 1×2 arc s. If the actual diameter of the lobe is θ arc s, then the equipartition magnetic field is $2.9 \times 10^{-4} \times \theta^{-6/7}$ G and the total energy content is $9.7 \times 10^{58} \times \theta^{9/7}$ erg. So far we have made no assumptions about the

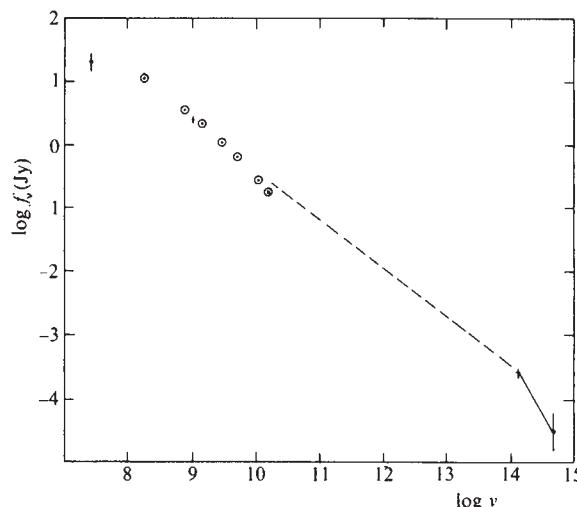


Fig. 1 Spectrum of 3C247W. Error bars are not shown when errors are $<10\%$. The dashed line joins the 5 GHz point and the IR point, and has a spectral index of -0.77 .

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mechanism responsible for the IR-optical emission, but the simplest assumption is that it is synchrotron radiation from very high-energy electrons¹. If this is so, then the electrons have energies up to at least $\gamma = 5.3 \times 10^5 \times \theta^{3/7}$, ($270 \theta^{3/7}$ GeV), and their synchrotron loss time is $540 \theta^{9/7}$ yr. These electrons must be continuously replenished by local particle acceleration and so the IR-optical emission locates the site of particle acceleration very accurately. Using the radio data, we can show that the alternative hypothesis, that the IR radiation is due to inverse Compton scattering of the microwave background by the relativistic electrons, is unlikely to be true in this source. The ratio of inverse Compton to synchrotron at IR wavelengths is^{1,20}

$$\frac{I_c}{I_s} = 1.6 \times 10^{-13} (7.5 \times 10^{-3})^{3-m} \times (T(1+z))^{(5+m)/2} B^{-(1+m)/2}$$

where T is the temperature of the microwave background, B is the magnetic field strength in the lobe, z is the redshift of the source and m is the index of the power law of the electron energy distribution. Taking $T = 2.9$ K (ref. 21), $z = 0.75$, and $B = 2.9 \times 10^{-4} \theta^{9/7}$ and $m = 2\alpha + 1 = 2.5$ from the radio data, we get

$$\frac{I_c}{I_s} = 7.3 \times 10^{-6} \times \theta^{-2.25}$$

Hence the observed IR emission cannot be due to inverse Compton scattering unless the lobe is unreasonably compact. At first sight, a total power output of 10^{46} erg s⁻¹ from the lobe seems rather high, but this is the natural consequence of extrapolating the radio spectrum to IR wavelengths. The eastern lobe of 3C265 (ref. 1) also has a luminosity of this order of magnitude if it has a spectrum similar to 3C247W, so 3C247W is not unique in having a large IR-optical luminosity. Where it is unusual is that the lobe has the same brightness in the R band as the parent Galaxy⁶. This result is independent of all the assumptions that we might make about the source, and the only way to avoid it is if the optical-IR source in the lobe is not physically associated with the radio galaxy, despite the low probability of this.

IR measurements are likely to prove to be a good way of detecting high frequency emission from radio lobes, since if an IR-optical spectral index of -1.7 is typical of radio lobes, then any lobe which has $m_B < 24$ will have $m_K < 18.9$, which can be detected in a few hours integration using UKIRT. Therefore it is possible that most radio lobes which can be detected optically can also be detected in the IR. It would be very useful if a selection criterion could be devised for making IR observations of radio sources which was based on the radio properties, as at present we are forced to select sources on arbitrary radio criteria, or be confined to those sources with known optical emission. In a radio selected sample consisting of 3C20, 3C47, 3C109, 3C153, 3C381 and 3C438 we failed to detect any emission brighter than 18th magnitude. An optically selected sample of 3C277.3, 4C26.42 and 4C29.30 have all shown emission at around the 17th magnitude level, and this will be reported elsewhere. Further work on this subject should help to increase our understanding of how particles are accelerated to high energies in the lobes; a subject which is rather poorly understood at present.

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Multifrequency observations of OV236 (1921-293) reveal an unusual spectrum

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As part of a programme of multifrequency single epoch observations of BL Lac objects and flat-spectrum radio sources we have examined the radio source OV236 (1921-293) which has been classified both as a quasar and a BL Lac object. Its polarization and variability at $2.2 \mu\text{m}$ and generally featureless optical spectrum are consistent with it being a BL Lac object. Wills and Wills² measured its optical polarization, variability and emission line spectrum and find it to have a redshift $z = 0.352$. It is an extremely variable radio source, during the outburst reported by Dent and Balonek³ it was the brightest 10-cm quasar in the sky. The outburst was first measured at 3.3 mm and was increasingly delayed at longer wavelengths; there was also evidence for correlated optical/radio variability during this outburst⁴. We have obtained J , H , K , 10, 20, 800 and $1,100 \mu\text{m}$ flux measurements of 1921-293 over a period of four days from the same telescope, enabling us to present a 'snapshot' of its IR to millimetre spectrum. The continuum spectrum of 1921-293 is suggestive of a peak in the submillimetre region. This excess could be due to an injection of high-energy electrons into a synchrotron emitting region, although thermal emission from dust remains a possibility.

The observations were made with the United Kingdom Infrared Telescope (UKIRT) on Mauna Kea over the period 10-13 September 1982. Although the conditions were stable during the run, the atmospheric water vapour was insufficiently low to allow $350\text{-}\mu\text{m}$ observations to be productive. In principle the special arrangement of focal plane instrumentation on UKIRT allows observations over the entire millimetre to $1 \mu\text{m}$ range to be carried out during one night. However, because precise calibration was required for our observations and the programme consisted of many sources, it was found to be more productive if no more than two instrument changes were made during one night. The $f/35$ Cassegrain focus was used for all observations; a rotatable dichroic being used to feed the separate detectors in the focal plane.

The millimetre and submillimetre measurements were made with the QMC-Oregon photometer system (P.A.R.A. *et al.*, manuscript in preparation) using narrow-band filters described by Cunningham⁵ and also shown in Robson⁶. FWHM beamwidths, obtained by scanning through Mars, were 58 and 65 arcs at 800 and $1,100 \mu\text{m}$ respectively. Other parameters of

the observations are given in Table 1. Calibration was performed against Uranus, with DR 21 being used to monitor the atmospheric extinction. The values of the brightness temperatures for Uranus were 94 ± 10 K and 84 ± 9 K at mean wavelengths of 1,070 and 770 μm respectively (C. T. Cunningham *et al.*, in preparation). The calibration procedure is described by Rowan-Robinson *et al.*⁷ and Cunningham *et al.*⁸. Because we used narrow-band filters, the assigned fluxes and wavelengths are rather insensitive to the value of the spectral index of the source.

The 10 and 20 μm measurements were made with the UKIRT common-user germanium bolometer. Calibration was made relative to Vega whose 10 and 20 μm fluxes were assumed to be 37 Jy and 12.0 Jy respectively. The near IR (*J*, *H*, *K*) measurements were made with UKT6, one of the UKIRT common-user InSb photometers. Calibration was made with respect to GL 811.1 whose *J*, *H*, *K* fluxes were taken to be 1.20, 1.35 and 1.05 Jy respectively, taken from ref. 9. It was also noted that at the time of observation the source appeared noticeably brighter than on our finding chart derived from the Palomar sky survey.

The results of our observations and calibration procedure are presented in Table 2 and Fig. 1. The errors quoted are purely statistical and we estimate our absolute calibration errors to be a maximum of 15%. Unlike other BL Lac objects and flat spectrum radio loud quasars (refs 10, 11, W.K.G. *et al.* and E.I.R. *et al.*, in preparation) the millimetre to IR continuum of 1921–293 is not a simple power-law. Instead the spectrum appears to rise at both 800 and 20 μm indicative of a peak in the submillimetre region (Fig. 1). There is also evidence of curvature in the near IR. The shape of the continuum spectrum of 1921–293 was different from the other BL Lacs in our sample observed at the same time. Although we regard the evidence for this possible excess as marginal, because of the known dramatic variability of this source we feel it is of sufficient potential importance to alert other observers.

Far IR excess in extragalactic objects is frequently attributed to thermal reradiation from heated dust¹². However, these sources are usually Seyfert-type or relatively local dusty galaxies. There is little direct evidence for dust in quasars or BL Lacs, although measurements are very difficult to make¹³. The submillimetre/far IR spectra of two nearby quasars, 1641+399 and 1226+023, show no evidence for reradiation from dust^{14,15}. The far IR excess emission attributed to heated dust generally peaks around 100 μm and has a FWHM of about 100 μm (see for example ref. 12). It is extremely difficult to see how thermal reradiation can explain the continuum spectrum of Fig. 1. Further measurements, particularly at submillimetre and far IR wavelengths, are crucial; any significant variability would rule out this mechanism.

The observed high polarization and variability in the near IR¹ is usually taken to be indicative of synchrotron emission. Attributing the overall radio to IR continuum to incoherent electron synchrotron mechanism, we can interpret a submillimetre peak as an outburst of radiation such as could be caused by a fresh injection of high energy electrons into a subsequently expanding synchrotron emitting plasma. This hypothesis is strengthened by the evolution of the 1979 outburst³ which was

Table 2 Observation results and calibration for source 1921–293

UT date Sept 1982	Mean wavelength (μm)	Flux	Standard error
10th	1,100	6.9 Jy	1.2 Jy
10th	800	12.3 Jy	2.4 Jy
12th	20	383 mJy	115 mJy
11th and 12th	10	90.4 mJy	15.5 mJy
13th	K	19.2 mJy	0.15 mJy
13th	H	14.8 mJy	0.21 mJy
13th	J	7.7 mJy	0.21 mJy

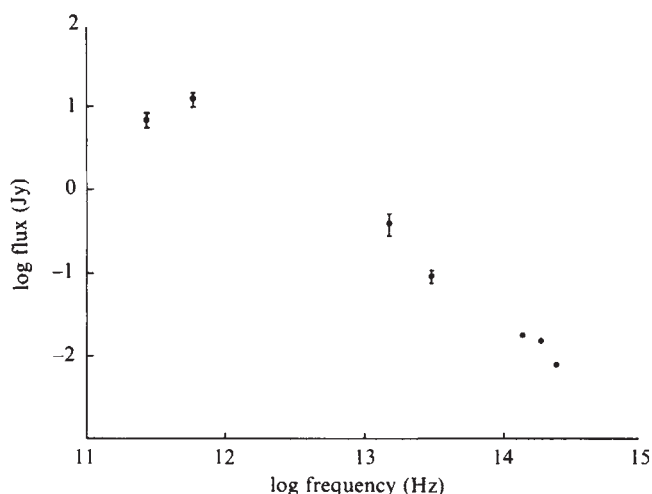


Fig. 1 The millimetre-to-IR spectrum of 1921–293 obtained over the period 10–13 September 1982 from UKIRT.

first observed at a wavelength of 3.3 mm. If we have observed the early stages of a similar outburst then we would expect further monitoring of 1921–293 at submillimetre and longer wavelengths to show the flux density to increase at progressively longer wavelengths over the next year. The curvature in the near IR may be interpreted as cutoff in the energy of the injected electrons^{16,17}.

Further observations and monitoring at all wavelengths of 1921–293 are necessary in order to determine the temporal evolution of the spectral shape. This will help to distinguish between different models of rapid variability in this type of source (for example, ref. 18). Monitoring of known variable sources at submillimetre wavelengths may prove to be a useful method of detecting outbursts at an early epoch.

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Table 1 Observational parameters for study of source 1921–293

Wavelength (μm)	Beam size (arc s)	Chopper throw (arc s)	Chopper frequency (Hz)
1,100	65	128	12.5
800	58	98	12.5
20	8	128	12.5
10	8	128	12.5
2.20	12.4	127	12.5
1.65	12.4	127	12.5
1.25	12.4	127	12.5

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Fast pulsars with disks

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It is quite likely that fast pulsars are not rare; there are several point-like radio sources with pulsar-like radio spectra that might prove to pulse in the millisecond range (J. H. Taylor, personal communication). The possibility that the recently discovered pulsar¹ PSR1937+214 is a new class of object has been considered by adherents of the vacuum model^{2,3}. We point out here that the measured parameters of PSR1937+214 are consistent with the proposed disk model of radio pulsars^{4,5}. Using the measured values of P and $\dot{P}$ and the scalings provided in this theory⁵, we expect a surface magnetic field strength of $\sim 10^8$ G, a radio-emission output of 10^{31} erg s⁻¹, and a weak (below detection with present instruments) output of energy in the X-ray and γ -ray ranges. The surface magnetic field cannot be checked, but the observed radio luminosity¹ is 3×10^{30} erg s⁻¹, in gratifying agreement with the theoretical expectation of the disk model.

In our model^{4,5}, an isolated magnetized rotating neutron star is encircled by a massive ($\sim 10^{-5} M_{\odot}$) fluid disk. Relative rotation between the disk and neutron star induces potential differences across the disk, and between the disk and neutron star as in a Faraday disk dynamo, permitting a return of the polar cap current from the disk to the star by auroral arcing. Pulsed emission arises from the return current flow to preferential regions of the star controlled by its surface magnetic field structure⁴.

In the disk model, the predicted average radio luminosity⁵ is related to P and $\dot{P}$ by

$$L_{\text{rad}} = 10^{30} \text{ erg s}^{-1} (\dot{P}/10^{-18}) (10/P)^{8/3} \quad (1)$$

where P is in milliseconds. The expected luminosity is 10^{31} erg s⁻¹ for 1937+214, compared with the observed value $\sim 3 \times 10^{30}$ erg s⁻¹. The position of 1937+214 relative to other pulsars is shown in Fig. 1, along with lines of constant radio luminosity from equation (1).

On average, pulsars have the luminosity indicated by equation (1), although there is considerable scatter (a factor of ~ 30 about the mean) as can be seen in Fig. 5 of ref. 5. Consequently, the close agreement in this case is probably fortuitous. It is difficult to compare this prediction directly against the vacuum rotator models owing to recent changes forced by the failure of the aligned rotator model⁶, and also the tendency to concentrate on explaining only the Crab pulsar⁷ rather than pulsars as a class. In any event, PSR1937+214 would be considered anomalously dim in the vacuum model, because only about 10^{-6} of the energy output appears as radio-emission, in contrast with the ratio of energy output to input of 10^{-3} typical of other pulsars⁸. As can be seen from Fig. 1, however, this pulsar has the luminosity expected by the disk model and is not anomalously dim.

According to the disk model, PSR1937+214 should not be a strong emitter of X rays and γ rays. The auroral zone potential drop, which gives the maximum potential available for accelerating particles, is 10^{10} V (see Fig. 4 of ref. 5). This is about average for pulsars in contrast to 10^{11} – 10^{12} V across the auroral zone for the Crab and Vela pulsars⁵. The energetic photon flux emitted by curvature radiation scales as particle

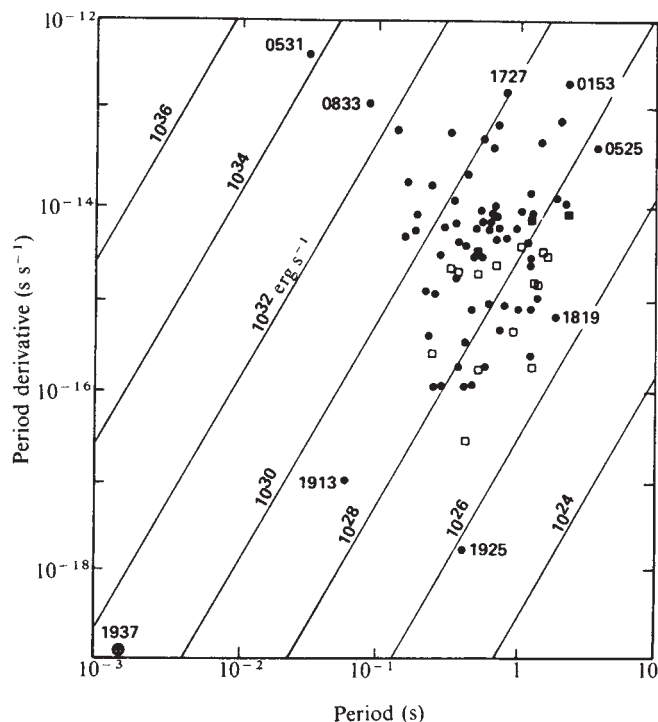


Fig. 1 Predicted radio luminosity of pulsars in the disk model (adapted from Fig. 3 of ref. 5).

energy to the fourth power, which may explain why only the Crab and Vela pulsars are bright sources of energetic photons.

In the disk model⁵, the surface polar magnetic field strength is given by

$$B_{\text{surf}} = 10^{10} \text{ G } (\dot{P}/10)^{1/2} (P/10)^{1/6} \quad (2)$$

For 1937+214, equation (2) indicates a surface field of $\sim 10^8$ G. We should emphasize that this is the value of B required to give the correct total power output from the neutron star, whereas equation (1) corresponds only to the radio power emitted.

Our magnetic field value of 10^8 G is slightly less than the 3×10^8 G inferred by Alpar *et al.*² and Arons³. In the disk model, the disk forces the field lines to open and form a stellar wind at the co-rotation distance (roughly 3 neutron-star radii) rather than at the light-cylinder distance (8 radii). Therefore, a given torque is produced in the disk model with a weaker surface field than in the vacuum models; the typical pulsar magnetic field is only 10^{10} G in the disk model compared with 10^{12} G in the vacuum models⁹. Consequently, a field of the order of 10^8 G is less surprising on the basis of the disk model, although it is an unusually weakly magnetized object. (We do not discuss here the issue of neutron star magnetization.)

We conclude that PSR1937+214 is not necessarily a new class of pulsar but that its properties are consistent with the disk model and do not require special explanation, although it may have had a peculiar evolutionary history¹⁻³.

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Transient X-ray rings around dwarf novae

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The possibility that point X-ray sources possess extended haloes arising from scattering of X rays by interstellar grains has been discussed by several authors (see, for example, refs 1, 2) and recent observations using the Einstein X-ray satellite have suggested that observational evidence for such haloes is now becoming available. For example, Rolf³ has shown that the X-ray source GX 339-4 has a discernible halo out to ~20 arc min, while Catura⁴ has argued that several low latitude X-ray sources may have haloes that extend to ≥ 10 arc min. Much of the theoretical work on such haloes to date has involved steady (time-independent) X-ray sources. While haloes around such sources can provide useful information about the scattering medium, variable X-ray sources are potentially much more powerful probes of the interstellar dust distribution^{5,6}. We discuss here the scattering of soft X rays emitted by dwarf novae in outburst, during which these objects undergo short (~1 day) eruption at soft X-ray wavelengths^{7,8}. We also consider the nature of the apparently transient soft X-ray 'ring' around the dwarf nova SU UMa (ref. 9) and we show that the properties of such a ring are consistent with those expected from the effect under consideration.

For simplicity, it will be convenient to assume that the soft X-ray light curve of a dwarf nova consists of a flat-topped pulse; the outburst luminosity in unit wavelength interval L_λ is taken to be black body at ~10⁶ K (ref. 7). The scattering of such a pulse by small interstellar grains gives rise to a soft X-ray ring that, owing to light travel time effects, is observed to expand and dissipate extremely rapidly after the dwarf nova itself is observed to erupt. Scattered X rays arriving at the observer at a time t after direct observation of the dwarf nova outburst are scattered from grains lying on an ellipsoid of revolution, with foci at source and observer⁶. For an X-ray pulse of finite duration, the scattering grains lie between two such confocal ellipsoids (see Fig. 1, which also defines the relevant parameters). Clearly, the detection of the resultant soft X-ray ring depends critically on the time of observation relative to that of the X-ray outburst. For example, if grains possessed a maximum scattering angle ϕ_1 ($\ll 1$ radian), the ring would expand from the central source in a time $9\phi_1^2 d/128c \sim 8$ days for a source at distance $d = 300$ pc and grains having $\phi_1 \sim 1^\circ$.

We suppose that the interstellar grain distribution between the source and observer is uniform and consists of spherical grains of radius a (as discussed by Rolf³ and Catura⁴, this is a good approximation for the case of X-ray scattering by grains having the usual distribution of sizes). The scattered X-ray intensity I_λ as a function of angular distance α from the central source is thus given by

$$I_\lambda(\alpha) = L_\lambda n_g d \int_{\theta_A(\alpha)}^{\theta_B(\alpha)} \frac{1}{4\pi r^2} \frac{d\sigma}{d\Omega} \exp\left\{-\left(\tau_1 + \tau_2\right)\right\} \frac{\sin \alpha}{\sin^2(\alpha + \theta)} d\theta \quad (1)$$

Here n_g is the interstellar grain number density, $r = r(\theta, \alpha)$ is the dwarf nova-grain distance, τ_1 and τ_2 are the extinction optical depths from nova to grain and grain to observer, respectively, and θ is the angle between the line of sight and the grain nova vector. For our present purposes we use the differential scattering cross-section of Hayakawa¹.

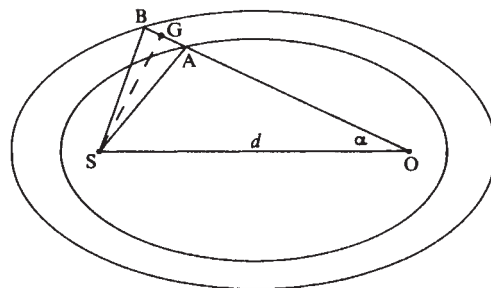


Fig. 1 Geometry for scattering of outburst X rays by interstellar grains; dwarf nova at S, observer at O. Observed X rays scattered from grains lying between two confocal ellipsoids. $\angle BSO = \theta_B$, $\angle ASO = \theta_A$, $\angle GSO = \theta$, $\angle AOS = \alpha$, $SO = d$.

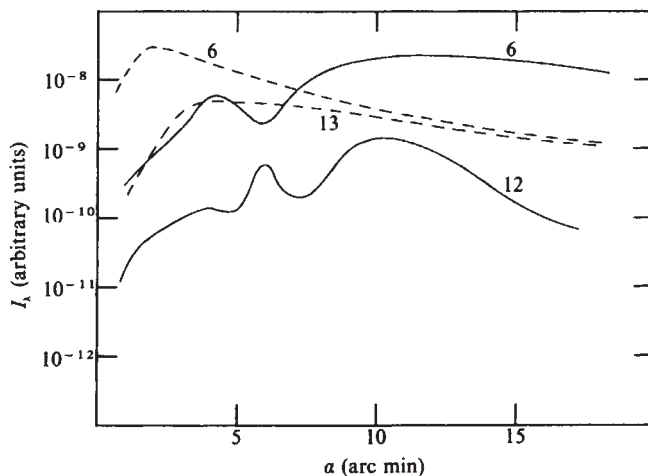


Fig. 2 X-ray intensity distribution around recently erupted dwarf nova; numbers are time, in days, from maximum. Broken curves, $a = 0.03 \mu\text{m}$, $n_g = 1.5 \times 10^{-11} \text{ cm}^{-3}$; solid curves, $a = 0.1 \mu\text{m}$, $n_g = 2.4 \times 10^{-12} \text{ cm}^{-3}$. Duration of soft X-ray pulse is 2 days in each case. Vertical scale arbitrary.

To quantify the effect under consideration we need to know the number density of interstellar grains. In the general interstellar medium, $n_g \approx 5 \times 10^{-13} \text{ cm}^{-3}$ (ref. 10), but this refers to those grains (possibly silicate) responsible for visual extinction ($a \sim 0.1 \mu\text{m}$) and does not necessarily represent the number density of smaller ($a \leq 0.05 \mu\text{m}$) grains which may be responsible for X-ray scattering. The UV spectrum of the dwarf nova SU UMa¹¹ shows the usual $\lambda 2,200$ extinction feature, as might be expected at a distance $d \sim 300$ pc (ref. 12). If this feature is due to small ($\leq 0.05 \mu\text{m}$) graphite grains, the implied grain density $n_g \sim 10^{-10} \text{ cm}^{-3}$, consistent also with abundance considerations. We have considered a range of grain radius and number density values and the results described below are typical.

The resultant intensity profile consists of an expanding ring, the rate of expansion of which seems to depend on grain size, being more rapid for larger ($\sim 0.1 \mu\text{m}$) grains than for smaller ($\sim 0.01 \mu\text{m}$). Some typical I_λ distributions are shown in Fig. 2 for various values of a and n_g , and for an X-ray pulse of duration 2 days. We note that the dimensions of the X-ray rings are ~ 10 – 20 arc min, and width ~ 10 arc min, for intervals of several days after outburst. Also, for larger grains, the intensity profile goes through a series of maxima and minima with respect to α ; this is because the condition $(4\pi a/\lambda) \sin(\phi/2) \ll 1$, where ϕ is the scattering angle, is no longer valid, that is, the scattering regime is anomalous diffraction rather than Rayleigh-Gans.

In the case of SU UMa, Cordova and Mason⁹ observed a ring of radius 14 arc min and width 6 arc min about 10 days after an eruption. Subsequent observations (F. A. Cordova and K. O. Mason, personal communication) failed to confirm the ring; thus, the possibility exists that instrumental effects may have been responsible, although the geometrical configuration

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of the ring suggests that background sources or statistical effects were unimportant. However, as discussed above, such a phenomenon would be consistent with the expected properties of an X-ray ring. Further support for this interpretation comes from the fact that the ring was detected as a soft, but not a hard, X-ray source⁹ and that the SU UMa system underwent an eruption shortly before the Cordova-Mason⁹ observation. Although no information is available on the X-ray behaviour of SU UMa during outburst, the soft X-ray fluxes of those dwarf novae that have been observed at X-ray wavelengths during optical activity increase by a factor ~ 100 (refs 7, 8). In the case of SU UMa, the required rise in soft X-ray flux may be estimated because the quiescent nova flux $f_{\text{nova}} (\approx 1.3 \times 10^{-11} \text{ erg s}^{-1} \text{ cm}^{-2})$ and the outburst halo flux $f_{\text{halo}} (\approx 6 \times 10^{-13} \text{ erg s}^{-1} \text{ cm}^{-2})$ are both known (ref. 9). For X-ray scattering, $f_{\text{halo}}^*/f_{\text{nova}}^* \approx f_{\text{halo}}/f_{\text{nova}}$, where an asterisk denotes outburst values. The expected flux in any X-ray ring may be computed by (i) assuming a value for the outburst flux f_{nova}^* at soft X-ray wavelengths, (ii) computing the resultant intensity distribution using equation (1) and assuming reasonable values of a and n_g , (3) integrating over the waveband of the Einstein imaging proportional counter detector and the width of the ring (see Fig. 2). For a range of values of n_g and a —similar to those used in deriving the results of Fig. 2—the increase in X-ray flux required, determined from assumption (i) and the known value of f_{nova} , is in the range ~ 10 – 20 . It seems likely therefore that the properties of the transient X-ray ring observed by Cordova and Mason⁹ are consistent with a modest rise in X-ray flux at outburst and a grain distribution that is consistent with other considerations.

The above discussion has been in terms of a uniform grain distribution. In reality, of course, the grain distribution is likely to be 'patchy' and such inhomogeneities will significantly affect the observed intensity profile. For example, the 'spur' noted by Cordova and Mason⁹ might be due to scattering of X rays by localized density enhancements in the interstellar grain population. More generally, dwarf novae—indeed any object that is subject to brief, predictable soft X-ray outbursts—could give rise to transient, expanding X-ray rings. Suitable observations of SU UMa and other dwarf novae using the imaging capability of the forthcoming Exosat satellite might be expected to reveal similar, short-lived X-ray haloes.

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Atmospheric storm explanation of saturnian electrostatic discharges

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The Voyager Planetary Radio Astronomy (PRA) experiments detected an impulsive (15–400 ms), broadband (20 kHz to 40 MHz) radio emission component that persisted throughout the two Saturn encounter periods^{1,2}. These bursts were grouped into episodes which recurred with a period of about 10 h 10 min, distinctly faster than the saturnian rotation period of 10 h 39.4 min (refs 1–5). This periodicity, coupled with the occurrence dependence on distance from Saturn, led Warwick *et al.*¹ to conclude that the bursts were related to the Saturn system

and coined the term SED for Saturn electrostatic discharges. We have analysed these SED episodes paying particular attention to their occurrence in both time and frequency as a function of spacecraft–Saturn geometry during the Voyager 1 encounter. We conclude that the characteristics of SED are best explained by a long-lived atmospheric lightning storm or systems of storms in Saturn's equatorial zone. From our model, we infer the ionospheric electron density near Saturn's noon meridian and over the night hemisphere.

Two SED source locations have been proposed¹ based on the repetition period: the atmosphere at equatorial latitudes where ground-based Doppler measurements⁶ and Voyager imaging measurements^{7,8} showed cloud-top wind velocities corresponding to a 10 h 10 min rotation period, and a discrete source in the rings at $1.8 R_s$ (R_s = Saturn radius = 60,330 km) where the keplerian revolution period equals 10 h 10 min. Atmospheric source locations were initially ruled out¹ because the ionospheric electron density measured by the radio science experiments on the Pioneer 11 and Voyager spacecraft^{9–11} was sufficient to prevent escape of emission much below 1 MHz, whereas SED were sometimes detected to frequencies as low as 20 kHz. Several authors then discussed the ring source alternative^{2–5}. However, Burns *et al.*¹² showed that many of the properties of SED are similar to lightning on other planets. They pointed out that the ring system casts a large shadow on the equatorial ionosphere, perhaps reducing the ionospheric electron density in this region enough to permit escape of low frequency atmospheric radio noise.

Our analysis of SED episodes provides new evidence to help resolve this controversy. Figure 1a summarizes the occurrence of SED as a function of time and frequency during the period immediately surrounding the Voyager 1 encounter. This diagram was constructed from an analysis of high-resolution dynamic spectrograms like those shown in Fig. 2, where the panel numbers correspond to the times indicated in Fig. 1. There are three crucially important features evident in Figs 1a and 2:

(1) The frequency extent of SED varies systematically with time. Before closest approach, SED are not observed below

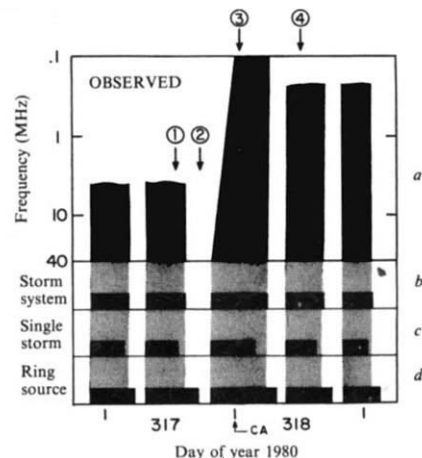
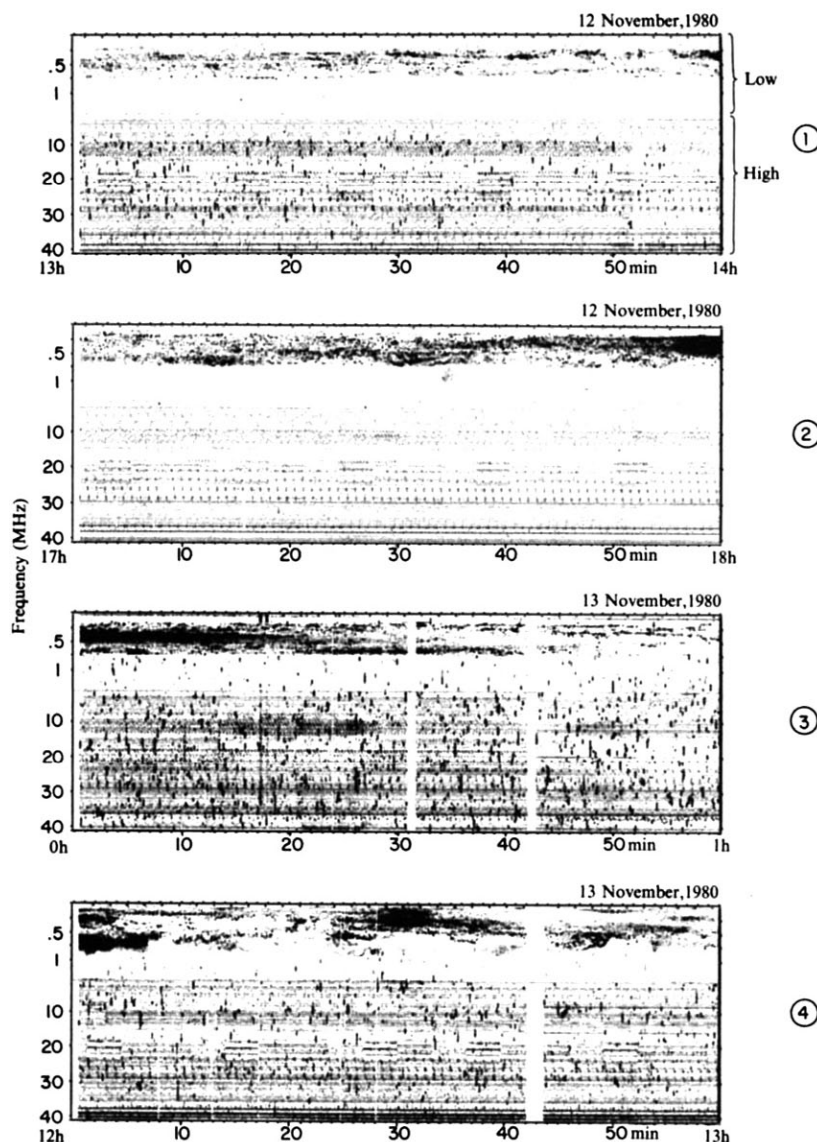


Fig. 1 Comparison of observed and predicted recurrence patterns for the five SED episodes centred on Voyager 1 closest approach. *a*, The times and frequencies where SED were detected (black) and not detected (white) by Voyager 1. Before and almost up to the time of closest approach (CA) no SED are seen below about 5 MHz. After CA, SED are regularly seen down to and even below 100 kHz. 1–4 correspond to data from panels 1–4 of Fig. 2. *b*, *c* and *d* compare the predicted recurrence patterns for a 60° wide atmospheric storm system, a single (point source) atmospheric storm, and a point source in the rings, respectively. The agreement between the observed and predicted start–stop times for the 60° wide surface storm *b* is clear. Note especially the coincidence between start and stop times in the case of the episode centred on CA, which lasts ~ 3 h longer than any other episode. The single-storm model (*c*) consistently predicts shorter episodes than those observed by ~ 2 h, and the ring source model (*d*) consistently predicts longer episodes by ~ 2 h.

Fig. 2 Four 1-h dynamic spectrograms of the highest resolution data from the PRA instrument. Panels 1–4 correspond to the arrows 1–4 of Fig. 1a. In these spectra, increases in intensity above the PRA receiver threshold are indicated by increases in pixel darkness. The SED are the short vertical streaks which appear in panels 1, 3 and 4. The grey variable background evident in the PRA high band is spacecraft-generated noise. The PRA instrument operates in two frequency bands. In the low band there are 70 1-kHz wide channels evenly spaced between 1.2 kHz and 1.3 MHz. In the high band, 128 200-kHz wide channels are evenly spaced between 1.5 and 40.5 MHz. The combined 198 channels are swept through every 6 s. In the low band, centred near 0.25 MHz, is the radio emission which emanates from Saturn's magnetosphere known as saturnian kilometric radiation^{1,2}.



~5 MHz (Fig. 2, panel 1). During the closest approach interval and for all episodes after closest approach, SED are detected to frequencies at least as low as several hundred kHz (Fig. 2, panels 3 and 4). Panel 4 of Fig. 2 shows data obtained at the same distance from Saturn as panel 1, but above the nightside. (2) There are very few detectable SED outside of the black bands in Fig. 1a. Panel 2 of Fig. 2, for example, shows a 1-h stretch of data between SED episodes with no SED observed above threshold.

(3) The appearances and disappearances of SED episodes are independent of frequency except for the onset of the episode centred on closest approach (CA). For this one onset, SED are first detected at the highest PRA frequencies near 40 MHz and gradually over a 4 h period fill the entire band. This last feature has been noted previously³.

The sparsity of SED between episodes strongly suggests that the main source region is being occulted once per revolution by the planet. The typical duration of the 'occultations' in Fig. 1a is greater than 3 h, or roughly one-third of the period of SED, even when the motion of the spacecraft past Saturn is taken into account. The pattern shown in Fig. 1a is inconsistent with a point source at $1.8 R_S$ undergoing consecutive occultations, because such an object would be out of view for only ~2 h or less. Figure 1d shows the occultation pattern that would result from an isotropically radiating point source in the B ring at $1.8 R_S$ phased so that its reappearance times match those in Fig. 1a. The black bands correspond to times when the source should be in view from Voyager and the intervals

in between are times when the source should be occulted by the planet. On the other hand, a point source in the equatorial atmosphere would be beyond Voyager's horizon for considerably more than 5 h unless SED are able to transmit over the horizon. This eliminates the possibility of a single localized atmospheric storm producing SED. Figure 1c shows the occultation pattern to be expected from a single isolated storm in the equatorial region.

It is difficult to modify the ring source theory so that all three of the previously mentioned observed parameters can be met. For example, an extended source in the rings would be occulted even less than a point source. We propose instead that the SED are generated by lightning from an extended storm system in Saturn's equatorial atmosphere. We determine the longitude of the leading edge of the storm from the reappearance times shown in Fig. 1a, and similarly the trailing edge from the disappearance times. Since this storm system moves at a rate different from the planetary rotation rate of 10 h 39.4 min, our determination of the storm's longitude in the Saturn longitude system (SLS) defined by Desch and Kaiser¹³ applies to a specific time. The storm's boundaries are 210° and 270° SLS for the leading and trailing edges at the epoch 15:10 (spacecraft time) on day 317 of 1980. The corresponding occultation pattern of this storm system is shown in Fig. 1b. Here, the black bands represent times when all or part of the 60° wide source region is within view of the spacecraft, and the intervals in between are times when the source region is completely occulted by Saturn. The remarkable degree of similarity between Fig. 1a and b,

contrasted with the lack of agreement with either Fig. 1c or d, strongly suggests that the disappearance and reappearance of SED is caused by occultation and reemergence of an extended equatorial storm system.

In addition to locating the storm in longitude, some restrictions can be placed on its latitudinal extent. If we assume that SED are generated at the cloud tops, the uncertainty² in the SED period of ± 5 min corresponds to an equatorial cloud top wind speed range of $\pm 80 \text{ m s}^{-1}$. Inspection of the Voyager 1 wind velocity profile deduced from the imaging data⁷ indicates that the storm must be centred at the latitude of peak wind which is in the vicinity of $+4^\circ$. The spread in latitude centred on $+4^\circ$ which corresponds to $\pm 80 \text{ m s}^{-1}$ is only about $\pm 2^\circ$. However, no wind velocity data are shown for the latitude band obscured from view by either the rings or the shadow of the rings, so that SED may emanate from that region.

The observed low-frequency cutoff can now be readily explained. Before encounter, when only SED above 5 MHz are observed, Voyager 1 was above the daylit hemisphere of Saturn, at a near-equatorial latitude and just duskward of local noon. The radio emission generated by the storm system had to propagate through the dayside saturnian ionosphere in order to reach the spacecraft. Since radio waves cannot propagate at frequencies below the electron plasma frequency, we propose that the low frequency cutoff of the SED is caused by high electron densities in the dayside saturnian ionosphere. The maximum electron density is estimated from $f_p = 9N_e^{1/2}$ where f_p is the electron plasma frequency (kHz) and N_e is the electron density (electrons cm^{-3}). Equating the 5 MHz cutoff to the plasma frequency, the implied maximum electron density in the ionosphere at the sub-spacecraft point is $3 \times 10^5 \text{ cm}^{-3}$. This is about an order of magnitude larger than the electron densities deduced from radio occultation measurements⁹⁻¹¹. However, those measurements were made near the dawn and dusk terminators, whereas the density derived here applies more nearly to the noon sector. This change in density from terminator to noon is comparable with the diurnal variation of electron density observed at Earth¹⁴. Only during and after the closest approach are the very low frequency SED observed. It is also during this period that Voyager observes the storms through the nightside ionosphere. This implies a maximum electron density in some portions of the nightside ionosphere of 100 cm^{-3} or less. Current theoretical models of Saturn's solar EUV-controlled ionosphere incorporate little or no diurnal variation of electron density, and fail to account for both the measured electron density and the altitude of the peak electron density¹⁵. However, Waite¹⁶ invokes a large diurnal altitude variation of Saturn's ionosphere to explain the differences in the vertical electron density profiles at the dawn and dusk terminators measured by the Pioneer 11 radio science investigation⁹. This would lead to a diurnal variation of electron density due to increased recombination of H^+ ions with CH_4 (the dominant loss mechanism) at low altitudes.

The frequency dependent onset of SED observed just before closest approach can now also be understood in terms of an atmospheric source. Figure 3 shows an equator plane projection of the Voyager 1 trajectory during the near encounter period; it also shows three views of Saturn and the storm system as seen from Voyager 1 at the indicated times. Inset a corresponds to the view about 1 h after the reappearance of SED when the leading edge of the storm system (fixed at the location described above) has just become visible. The radio emission accompanying this storm system propagates through a long slant path in the ionosphere when it first appears on the observer's horizon resulting in considerable refraction. We have performed ray tracing analyses for ionospheric densities of 10^4 – 10^5 cm^{-3} which demonstrate that high frequencies ($>30 \text{ MHz}$) are able to propagate directly to the spacecraft even when the storm is on the limb, whereas lower frequencies are refracted away from the line of sight. As the planet rotates, the path from the leading edge of the storm to Voyager travels through progressively less ionosphere so that less refraction occurs permitting lower and

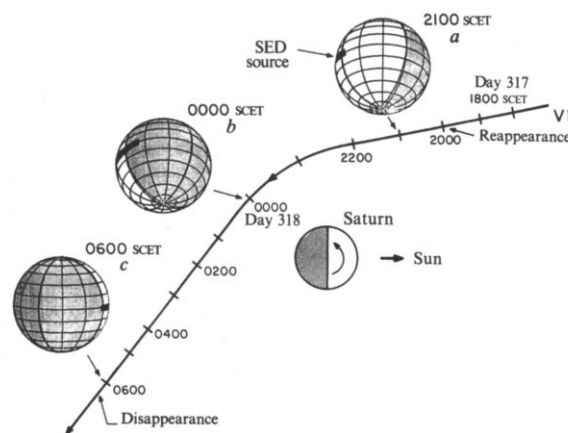


Fig. 3 Voyager 1 trajectory past Saturn is shown projected into the equatorial plane. The shaded globes show the planetary aspect and SED source location as viewed by Voyager 1 at three times near closest approach. On day 317 at 1950 SCET, the source reappears on the west limb of the planet, marking the onset of the third episode in Fig. 1. At 2100 SCET (a), the leading edge of the source is about 30° beyond the west limb; at 0000 SCET (b), the leading edge is about 50° beyond the limb and well into the nightside hemisphere, permitting escape of very low frequency ($<1 \text{ MHz}$) SED; by 0600 SCET (c), the trailing edge of the source is near the eastern limb, close to disappearing beyond the spacecraft horizon. At 0625 SCET the episode ends as the source disappears from Voyager's view.

lower frequencies to be observed. This frequency dependence of SED onset times should occur to some extent for all reappearances and disappearances; however, this effect is observable only for the occasion near closest approach. Since the storm system rotates every 10 h 10 min, the angular velocity is such that in 20 min the storm has moved off the limb by nearly 12° . This is enough to permit even the lowest frequencies (5 MHz) to be observed and corresponds to an onset drift rate of about 1.5 MHz min^{-1} . A drift rate this large is not discernible in the SED data due to the sparsity of SED near the reappearance and disappearance times and the complicated, frequency-dependent sensitivity pattern of the PRA instrument. However, during the period near closest approach, the Voyager 1 spacecraft was moving at a rapid enough speed around the planet to nearly match the planetary rotation speed. Thus, the SED source spent a much longer time travelling across the visible face of Saturn than during any other episode, long enough to generate the observed 0.2 MHz per minute onset drift rate.

Inset b of Fig. 3 shows the first time that Voyager was able to view the storm on the dark side of the planet. This is also the time when SED were first detected down to low frequencies, indicating a very low ionospheric electron density.

The SED occurrence statistics during the closest approach episode also support our interpretation that an extended storm region is responsible for the emission. We have counted the number of SED per unit time in the 30–40 MHz frequency range, where ionospheric refraction is unimportant, and have converted these counts to the equivalent occurrence rate of SED. At the beginning of the closest approach episode (near 20 h on day 317), SED occur at a rate of one every 20–30 s. Slowly over the succeeding 4-h interval, the SED occurrence rate rises to a value of 1 every 5 s. This occurrence behaviour is yet another indication that successively more and more of an extended SED source region is becoming visible to the spacecraft, until the entire storm system is visible.

We believe that the PRA observations of SED can contribute to the study of Saturn's atmosphere and ionosphere. Our proposed storm model for the source of SED explains the occurrence pattern and frequency-dependence of SED and does not invoke exotic or unexplained physical phenomena required by the ring source theories.

We thank J. H. Waite Jr for discussions about Saturn's ionosphere, and J. W. Warwick, D. R. Evans and J. H. Romig for suggesting that the extended source size should be evident in the occurrence rate of SED in the high-frequency range observed during the closest approach episode.

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Gravitational anisotropies of gyromagnetic ratios and tests of general relativity

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It is a direct consequence of the Einstein equivalence principle (EEP) that all atomic clocks will run at the same rate if situated at the same point in space-time. This prediction of a universal gravitational redshift is a property of any self-consistent metric theory of gravity¹. The class of experiments considered here essentially involves a comparison of two clocks, one of which is a quartz crystal stabilized to a caesium-beam atomic frequency standard, and the other, more important for this discussion, is a NMR clock, for which the 'ticks' are provided by the free precession of a sample of polarized nuclear spins in a stable and uniform magnetic field. Two experimental null results have been reported, one by Hughes² and the other by Drever³. We point out that some measurements already exist for spin 1/2 systems which can put tighter limits on anisotropic precession frequencies than the Hughes–Drever results, and we suggest an experimental technique that should allow a further improvement in precision by up to six orders of magnitude. Apart from the interest to gravitation physics these results are also important for high precision metrology and the provision of standards of magnetic flux density and electric current.

Several different ways have been proposed by which NMR clocks might violate EEP. Cocconi and Salpeter⁴ suggested that inertial mass might exhibit anisotropy, reflecting the anisotropic distribution of mass-energy in the universe, in the spirit of Mach's principle. If the nuclear spin species possesses a non-spherical mass and charge distribution ($s > 1/2$) the degeneracy of the Zeeman splittings will be lifted, reflecting the difference in electrostatic energy of the magnetic sub-levels. Dicke⁵ and Will¹ have interpreted the results of the Hughes–Drever experiment in a rather different way, regarding the null result as demonstrating the isotropy of space or the exactness of local Lorentz invariance. Any non-zero result would then give evidence for a long-range tensor field coupling directly to mass, in addition to the metric term of general relativity. Other authors have proposed that EEP might be violated by a direct coupling between gravity and the spin of a particle^{6,7}.

Any observed frequency shift might arise from a number of sources, for example, a preferred frame at rest with respect to the 2.7 K radiation, or a nearby mass distribution such as the Galaxy, the Sun, or the Earth. Imagine, as an example of the kind of interaction which such experiments can explore, that

the gravitational interaction is not symmetric under parity or time reversal. Take a particle with mass m and spin s , where the gravitational field results from the presence of a large mass M at a distance R . The simplest form of interaction which would exhibit non-conservation of both parity and time reversal, while still maintaining overall parity and time invariance, is of the form⁶ $U(\mathbf{s} \cdot \mathbf{r})$ where $\mathbf{r}$ is the unit vector directed from the large mass to the particle. These assumptions lead to a term being added to the gravitational potential energy of the particle, of the form

$$\phi_G = U_0 + \delta U_0 = U_0[1 + A \mathbf{s} \cdot \mathbf{r}] \quad (1)$$

where U_0 is the usual newtonian term, GMm/R , so that A is a measure of the degree of breakdown of parity and time invariance⁶. As with the previous proposals the effect would show up as a variation of the NMR frequency with the relative orientation of the axis of quantization.

Several experiments have been performed in which the eigen-frequencies of a nuclear spin system have been examined as the relative orientations of the magnetic and gravitational fields have been changed, either directly, by rotating the magnetic field with respect to the surface of the Earth, or indirectly, by allowing the Earth's rotation to bring about the variation. In the latter case the caesium frequency may also be affected, but this is expected to be many orders of magnitude smaller and could be verified by separate experiments. The consistency of the individual readings of spin precession frequencies made at a particular site and their independence of sidereal or solar time allows limits to be set on the gravitational effects of the Sun or Galaxy and hence to set limits on the anisotropy of space and inertia with respect to the Sun or to the Galactic centre. The gravitational effect of the Earth may be studied by making measurements with the axis of the magnetic field in the horizontal and vertical direction. The effects are best studied in weak magnetic fields and low-field NMR measurements have provided the most sensitive detectors of energy shifts, with a sensitivity of at least 10^{-17} eV.

The earliest², and most accurate limit set hitherto³, involved carrying out a measurement of the free precession frequency of a sample of ^7Li nuclei in the Earth's magnetic field. By choosing a nuclear spin system with $s > 1/2$, so that the Zeeman splitting of the magnetic sub-levels would be affected differently by the interaction described in equation (1), it was not necessary to maintain the magnetic field constant, but only to look for beat signals between the observed NMR frequencies from the various Zeeman levels. In this way Drever was able to show that the frequency difference between these levels was < 0.02 Hz.

Several attempts have been made to observe the NMR frequency of a nuclear spin system in which the magnetic field was maintained constant as the apparatus was tilted from the vertical to the horizontal position. Although a relatively large effect has been claimed⁸, subsequent, and more accurate work, showed no effect with a precision of around 0.2 Hz (ref. 9). There are two major problems with this approach. First the stability of the external field, even in quiet magnetic environments, limits the overall precision to around 1 mHz. Second, movement of the apparatus with respect to the gravitational field inevitably leads to dimensional distortion at some level of precision, with a possible consequent change in B .

Improved limits may be obtained by combining measurements of the gyromagnetic ratio of the proton, γ_p , made in horizontal and vertical magnetic fields. The most precise measurement of γ_p to date is that by Olsen and Williams at the National Bureau of Standards¹⁰ who made measurements over at least 2 yr. The precession frequency in their horizontal solenoid was ~ 20 kHz and this was measured with an uncertainty of one part in 10^7 , or 2 mHz. Their result may be combined with the two determinations of γ_p which have used a vertical solenoid. These are the preliminary National Physical Laboratory (NPL) result¹¹ and the result at National Institute of Metrology, Peking¹² with free precession frequencies of

~50 kHz and 20 kHz respectively and a quoted frequency resolution of 2.5 mHz.

The values of γ_p derived from these three experiments agree at the 4 p.p.m. level, or equivalently 80 mHz (although the two vertical measurements seem to differ significantly below this level—probably for metrological reasons). Thus we may immediately put an improved limit on the gravitational dipole moment of the proton, namely that the difference between the proton energy when its spin axis and the direction of the external gravitational field are parallel and when they are orthogonal is $\leq 5 \times 10^{-36}$ J. Of course, using measurements made in different laboratories and on three continents is far from the ideal method of looking for a horizontal-vertical difference in gyromagnetic ratios, but it illustrates the high precision required in modern metrology and underlines the importance of establishing improved limits by other means.

In this connection, a measurement of the longitudinal relaxation time of optically pumped ^3He in a very low ambient field¹³, showed that the phase coherence of the signal persisted for at least 11 h. It can be inferred from this reference that the precession frequency varied during this time by not more than ~100 μHz .

Several novel techniques in superconducting metrology can be used to improve significantly on the existing limits established with room temperature NMR systems. An experiment in progress¹⁴ uses a stable magnetic field of the order of 10^{-4} T trapped through a superconducting tube. The quantization of magnetic flux linking the tube, together with the extremely high isolation against external magnetic field changes provided by the tube, means that the flux density can be maintained uniform and constant to $< 10^{-13}$ T over many hours. The free precession frequency of a small sample of normal liquid ^3He will be measured in this stable environment, using a SQUID (superconducting quantum interference device) detector, to provide very high signal-to-noise ratios, independent of frequency. Results so far suggest that with the present system it will be possible to measure the ^3He free-precession frequency with a precision of 1 μHz , and that a redesign of the equipment to enable a quantum-limited SQUID to be used could lead to a sensitivity of ~1 nHz. The frequency, relative to that of a quartz crystal standard which is in turn fixed relative to a caesium standard, will be measured as a function of sidereal time. It should also be possible to tilt the apparatus in the Earth's gravitational field. Earlier attempts have been limited by unknown dimensional distortions, but in this experiment a microwave resonance dimensional measuring technique¹⁵ will allow the cross-sectional area of the tube to be monitored with very high precision.

For the two possibilities discussed above the fractional anisotropy $\delta m/m$, of the nuclear inertial mass would have the following angular dependencies as Legendre polynomials:

$$\begin{aligned}\delta m/m &\sim AP_1(\cos \theta) \\ &= A \cos \theta\end{aligned}\quad (2)$$

for a parity non-conserving direct coupling between the nuclear spin and the gravitational field, and

$$\begin{aligned}\delta m/m &\sim AP_2(\cos \theta) \\ &= A(3 \cos^2 \theta - 1)/2\end{aligned}\quad (3)$$

for a long-range non-metric field¹⁶. In each case the free precession frequency will show a similar angular dependence as the inertial mass, and in both cases the parameter A measures the strength of the EEP violating part of the gravitational interaction compared with the dominant metric contribution.

Table 1 summarizes the upper limit set on the parameter A with respect to the three sources of gravitational field for existing and for the projected work described here (with the reference and the assumed frequency limits given in parentheses). Table 1 shows that the results discussed here set tighter limits on the isotropy of the gravitational interaction for spin

Table 1 Upper limit on parameter A

Source of gravitational field	Best previous limit on A (Hz)	Improved limit based on work already published (mHz)	Predicted precision of ^3He SQUID magnetometer (μHz)
Earth	1.3×10^{-15} (± 0.2)[9]*	5×10^{-16} (± 80)[10–12]*	2.1×10^{-21} (± 1)[14]*
Sun	1.3×10^{-18} (± 0.02)[3]†	1.6×10^{-20} (± 0.1)[13]*	1.6×10^{-22} (± 1)[14]*
Galaxy	1.4×10^{-20} (± 0.02)[3]†	1.7×10^{-22} (± 0.1)[13]*	1.7×10^{-24} (± 1)[14]*

Values in square brackets refer to reference where work is described. Values in parentheses indicate precision of frequency measurement.

* Spin 1/2.

† Spin 3/2.

1/2 particles than hitherto. They also suggest that it would be worth analysing the raw experimental data in published work^{10–13} for time variations of the spin precession frequency. Although the interpretation of the results might be more difficult, we include here measurements of the type in which the effects of any magnetic flux changes are removed by monitoring the differences between the spin precession frequencies of various atomic species. Thus, Karwaki¹⁷ has reported that short-term drift rate sensitivities of 0.1°h^{-1} have been achieved with this type of NMR gyroscope. Once a null limit has been set for spin 1/2 particles they may obviously be used as references for other particles. Tests of other aspects of general relativity based on different forms of interaction are also possible.

Apart from their utility in the fields of cosmology and fundamental physics, the upper limits given in Table 1 are barely satisfactory for present-day metrological measurements in weak magnetic fields^{18,19} (and therefore might have an effect on the accuracy with which the unit of magnetic flux density can be realized), or for applications involving nuclear gyroscopes^{17,20}. We emphasize that, as with all null experiments, the interpretation of the results of this type of measurement in terms of their implications for fundamental theories, such as tests of Mach's principle²¹ and of EEP²², requires a very careful examination of the physical assumptions that are implicit in the method of making the measurements. We conclude that there is a need for a further examination to be made of the theoretical and experimental evidence for the isotropy of gyromagnetic effects, both for spin 1/2 particles and for particles with spin greater than this, and that there is now the technological capability available for improving on the present experimental limits.

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Growth rate of a vesicomid clam from the 21° N East Pacific Rise hydrothermal area

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Previously, we determined the growth rate of a vesicomid clam from the Galápagos spreading centre by using natural radionuclide analyses of serially sampled shell layers¹. The growth rate was $\sim 4 \text{ cm yr}^{-1}$ measured along the 12 cm length of the axis of maximum growth, yielding an age for the clam of 3–4 yr, assuming this rapid rate for the entire age of the clam. We noted that the linear growth rate was actually highest in the last year of the life of the organism; this could indicate a change in food supply, which in turn, in this chemically driven ecosystem, would be an index of hydrothermal activity. We have now determined the growth history of a vesicomid clam from the 21° N East Pacific Rise (EPR) site using ^{228}Ra , ^{226}Ra and ^{228}Th measurements described earlier¹. The ^{210}Pb (half life 22.3 yr)– ^{210}Po (half life 138 days) couple is useful only for 2 yr, at which time secular equilibrium is attained, whereas ^{228}Ra (half life 5.7 yr) and its progeny, ^{228}Th (half life 1.9 yr), provide information on a longer time scale. The $^{228}\text{Th}/^{228}\text{Ra}$ activity ratio reaches the transient equilibrium value of 1.5 in ~ 10 yr and the ^{228}Ra decay can be followed for ~ 30 yr. We report that the average growth rate along the axis of maximum growth (10 cm) is 0.27 cm yr^{-1} resulting in an estimated age of 27 yr for the clam, about 10 times older than a clam of comparable size from the Galápagos spreading centre. There is evidence for changes in growth rate with time.

The 21° N EPR site has been described elsewhere^{2,3}. Our sample was collected live by H. Craig of the Scripps Institution of Oceanography, La Jolla, California, and by R. Hekinian of CNEX, Brittany on the 1979 *Alvin* expedition to the 21° N EPR site. The organism was shipped to us in dry ice soon after recovery and we sampled one valve of the shell as described earlier¹ except that now we were able to sample at closer intervals than before. The more recent parts of the shell were sampled at 0.5-cm intervals. The older parts provided sufficient material for analysis only with larger sampling intervals. The clam was 15.7 cm long and 10 cm along the axis of maximum growth, the line along which all our measurements are referred.

Table 1 shows the analytical data. We have followed the convention of representing activity ratios by parentheses. The ^{228}Ra and ^{228}Th values listed have been corrected for activities supported by ^{232}Th by assuming initial activity ratios of unity for $(^{228}\text{Th}/^{232}\text{Th})$ and $(^{228}\text{Ra}/^{232}\text{Th})$. The ^{226}Ra concentration does not vary greatly over time (range $\sim 25\%$) but there is significant variation in the ^{210}Pb concentration (by a factor of 2). We observed the same feature in the Galápagos spreading centre clam but with an even smaller range for ^{226}Ra . Difficulties with the ^{208}Po spike calibration vitiated our results for ^{210}Po but because of the slow growth rates we encountered (see below) the results would not have been very useful anyway.

There are significant variations in $(^{228}\text{Ra}/^{226}\text{Ra})$ and $(^{228}\text{Th}/^{228}\text{Ra})$ with the former decreasing away from the growing margin and the latter increasing to values which are indistinguishable, within the analytical uncertainty, from the transient equilibrium value of 1.5. Both these ratios serve as chronometers to constrain the growth rate of the clam. The

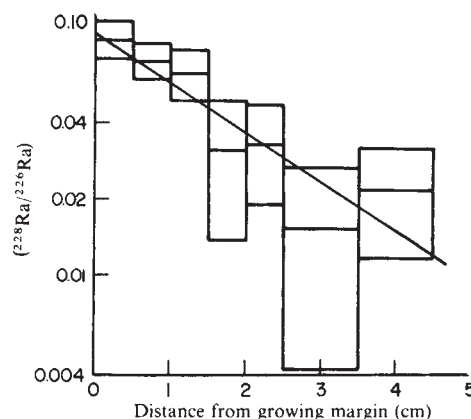


Fig. 1 $(^{228}\text{Ra}/^{226}\text{Ra})$ as a function of distance from the growing margin of a 21° N EPR vesicomid clam. The average growth rate is 0.27 cm yr^{-1} with an initial $(^{228}\text{Ra}/^{226}\text{Ra})$ of 0.09. ^{228}Ra measurements have been corrected for ^{232}Th supported activity as described in the text.

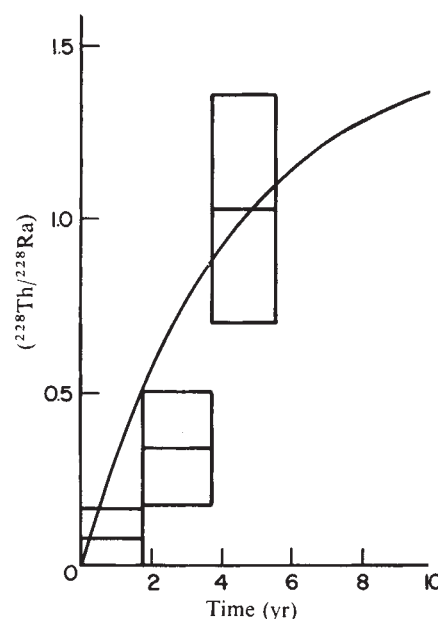


Fig. 2 $(^{228}\text{Th}/^{228}\text{Ra})$ data for the three youngest 0.5-cm thick layers plotted on a $(^{228}\text{Th}/^{228}\text{Ra})$ growth curve assuming no initial ^{228}Th and a linear growth rate along the axis of maximum growth of 0.27 cm yr^{-1} .

simplest approach is to determine the average growth rate of the clam using the $(^{228}\text{Ra}/^{226}\text{Ra})$ chronometer. We assume, as a first approximation, that $(^{228}\text{Ra}/^{226}\text{Ra})$ at the time of incorporation in each shell layer has remained constant with time, even though this was not strictly the case for the Galápagos vesicomid¹. Figure 1 shows the $(^{228}\text{Ra}/^{226}\text{Ra})$ variation with distance from the growing margin (from 0–4.5 cm); we have not included the 4.5–5.5 cm sample because of the large uncertainty in $(^{228}\text{Ra}/^{226}\text{Ra})$. A best-fit line yields a rate of $0.27 \pm 0.12 \text{ cm yr}^{-1}$ and an initial $(^{228}\text{Ra}/^{226}\text{Ra})$ of 0.09. With an average growth rate of 0.27 cm yr^{-1} along the axis of maximum growth over the lifetime of the clam, the age of the clam is 27 yr, about 10 times older than the clam from the Galápagos spreading centre¹.

The $(^{228}\text{Th}/^{228}\text{Ra})$ chronometer can be used as a check on the reliability of this growth rate estimate. It can provide information only on the three sequential samples from the growing margin inwards (0–1.5 cm) as the transient equilibrium value of 1.5 is reached (within error) in the 1.5–2.0 cm interval. Proceeding from the growing edge inwards, $(^{228}\text{Th}/^{228}\text{Ra})$ increases. Figure 2 shows the curve constructed using the Bateman equation describing the ingrowth of ^{228}Th from ^{228}Ra

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Table 1 Uranium and thorium decay series nuclides in sequential layers of a vesicomyid clam from 21° N East Pacific Rise hydrothermal vent

Interval from growing margin (cm)	Sample wt (g)	$^{226}\text{Ra}^*$	^{210}Pb	^{232}Th	$^{228}\text{Ra}^\dagger$	$^{228}\text{Th}^\dagger$
a, Specific activity at time of collection (d.p.m. g⁻¹ of CaCO₃)						
0–0.5	7.05009	0.0798	0.747 ± 0.031	0.0012 ± 0.0003	0.00682 ± 0.00108	0.00054 ± 0.00059
0.5–1.0	6.33523	0.0744	0.541 ± 0.029	0.0012 ± 0.0003	0.00524 ± 0.00080	0.00178 ± 0.00081
1.0–1.5	3.8774	0.6851	0.635 ± 0.045	0.0011 ± 0.0004	0.00430 ± 0.00094	0.00443 ± 0.00104
1.5–2.0	3.21726	0.0664	0.406 ± 0.019	0.0021 ± 0.0005	0.00207 ± 0.00115	0.00360 ± 0.00104
2.0–2.5	2.59408	0.0754	0.456 ± 0.026	0.0010 ± 0.0005	0.00247 ± 0.00104	0.00684 ± 0.00187
2.5–3.5	2.93286	0.0951	0.323 ± 0.015	0.0012 ± 0.0004	0.00145 ± 0.00105	0.00386 ± 0.00114
3.5–4.5	2.40043	0.0986	0.642 ± 0.037	0.0010 ± 0.0004	0.00216 ± 0.00100	0.00382 ± 0.00126
4.5–5.5	1.44693	0.0751	1.006 ± 0.064	0.0017 ± 0.0005	0.00165 ± 0.00206	0.00097 ± 0.00139
b, Activity ratios						
	$(^{210}\text{Pb}/^{226}\text{Ra})$		$(^{228}\text{Ra}/^{226}\text{Ra})$		$(^{228}\text{Th}/^{228}\text{Ra})$	
0–0.5	9.36 ± 0.61		0.0854 ± 0.0142		0.079 ± 0.088	
0.5–1.0	7.28 ± 0.53		0.0704 ± 0.0113		0.340 ± 0.163	
1.0–1.5	9.26 ± 0.80		0.0628 ± 0.0141		1.030 ± 0.331	
1.5–2.0	6.12 ± 0.42		0.0312 ± 0.0175		1.733 ± 1.087	
2.0–2.5	6.05 ± 0.46		0.0328 ± 0.0139		2.764 ± 1.386	
2.5–3.5	3.40 ± 0.23		0.0152 ± 0.0110		2.660 ± 2.072	
3.5–4.5	6.51 ± 0.50		0.0219 ± 0.0102		1.771 ± 1.003	
4.5–5.5	13.40 ± 1.08		0.0220 ± 0.0275		0.586 ± 1.112	

* Average error estimated at 5% c.v.

† ^{232}Th -corrected values assuming $(^{228}\text{Ra}/^{232}\text{Th})$ and $(^{228}\text{Th}/^{232}\text{Th})$ are both at unity at time of incorporation.

assuming no initial ^{228}Th . The $^{228}\text{Th}/^{228}\text{Ra}$ activity ratio for each sample is plotted assuming a linear growth rate of 0.27 cm yr⁻¹ based on the $(^{228}\text{Ra}/^{226}\text{Ra})$ chronometer. Within the errors the $(^{228}\text{Th}/^{228}\text{Ra})$ data are compatible with the $(^{228}\text{Ra}/^{226}\text{Ra})$ mean linear growth rate estimate.

We have used the individual $(^{228}\text{Th}/^{228}\text{Ra})$ measurements to calculate ages for the three samples from the growing margin to 1.5 cm. (At an average growth rate of 0.27 cm yr⁻¹ this is the limit of disequilibrium.) The calculation assumes a constant linear growth in each interval and assigns the measured $(^{228}\text{Th}/^{228}\text{Ra})$ to the midpoint of each interval. This procedure yields ages of 0.22, 1.1 and 4.9 yr for the midpoints of the three intervals and, with the constant linear growth assumption, these correspond to times of deposition of 0.44 yr for 0–0.5 cm, 1.4 yr for 0.5–1.0 cm and 6.3 yr for 1–1.5 cm. Using these values we calculate the growth rates to be 1.1 cm yr⁻¹ for 0–0.5 cm, 0.36 cm yr⁻¹ for 0.5–1.0 cm and 0.08 cm yr⁻¹ for 1.0–1.5 cm. We have estimated the uncertainties on these values by calculating upper and lower limits using the 1σ errors on the individual ^{228}Th and ^{228}Ra activities. This approach gives ranges in the times of deposition of 0–1.2 yr for 0–0.5 cm, 1.0–1.8 yr for 0.5–1.0 cm and 2.8 to ∞ yr for 1.0–1.5 cm. The corresponding growth rates for the intervals are 0.42 to ∞, 0.28–0.50 and 0–0.18 cm yr⁻¹ for 0–0.5 cm, 0.5–1.0 and 1.0–1.5 cm, respectively. These results suggest that either the growth rate or the initial $(^{228}\text{Th}/^{228}\text{Ra})$ has varied during the life of the clam.

Although our data do not definitely demonstrate variable growth rates with time, they can be interpreted in this manner. Support for this suggestion comes from our ^{226}Ra results. There is a positive correlation between the calculated differential growth rate and the ^{226}Ra concentration of the shell. As hydrothermal waters are higher in ^{226}Ra than ambient seawater at the 21° N EPR site⁴, the greater the hydrothermal flux to the vesicomyid field the greater the ^{226}Ra concentration of the water and the higher the chemisynthetic rate, which presumably determines the growth rate of the clam. As ^{226}Ra and Ba correlate in the hydrothermal waters⁴ it may be possible to relate the Ba concentration of a shell layer to hydrothermal activity. If this is the case, we may be able to obtain a record of vent activity at any particular hydrothermal field by a serial analysis of a shell for such a diagnostic element.

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Cretaceous and Tertiary fossils in glacio-marine strata at Cape Melville, Antarctica

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Glacio-marine sediments of the Cape Melville Formation (King George Island, South Shetland Islands, West Antarctica) contain a rich and predominantly invertebrate fauna. The occurrence of nannoplankton and belemnites might be used as evidence for dating the glacio-marine Cape Melville Formation as Cretaceous. However, the co-occurrence of the coral genus *Flabellum* (Eocene–Recent) and the foraminiferal genus *Uvigerina* (Eocene–Recent) indicate that recycling of the nannoplankton and belemnites has occurred. The sediments of the Cape Melville Formation contain a variety of glacially striated ice-rafted dropstones (up to 2 m) of Antarctic continent provenance. These sediments lie between lavas of the Sherratt Bay Formation and Pliocene Polonez Cove Formation, and may correlate with a late Miocene glaciation of Antarctica.

Cape Melville forms the easternmost extremity of King George Island in the South Shetland Islands, West Antarctica (Fig. 1). Before the Polish geological and palaeontological investigations of 1980–81, the only information available on the geology and structure of the area indicated the presence of layered volcanic rocks of Plio-Pleistocene age^{1–4}, together with a poorly preserved bivalve *Malletia* sp., said to resemble similar forms from Miocene strata of southern South America⁵.

Our field work^{6–8} allowed us to distinguish two lithostratigraphical units of group rank: the younger Penguin Island Group represented by Quaternary volcanics of the extinct Melville Peak cone; and the older Moby Dick Group consisting of a pre-Quaternary sequence of effusive volcanics at the base, succeeded by shallow-marine, and finally by slightly deeper-water highly fossiliferous glacio-marine sediments, all intruded by numerous basaltic and andesitic dykes (Figs 2, 3).

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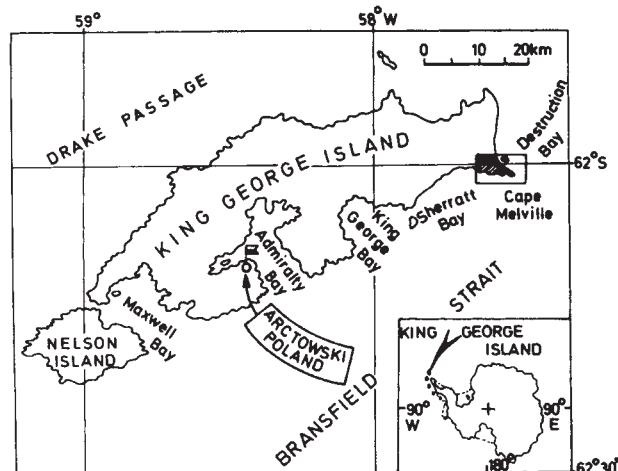


Fig. 1 Position of Cape Melville in King George Island and in Antarctica (inset).

The Moby Dick Group has been subdivided into three formations⁶⁻⁸. The lower Sherratt Bay Formation consists of an olivine-augite basalt lava sheet >60 m thick, extruded in sub-aerial conditions. There are no indications of either submarine or glacial conditions associated with this eruption. The lava is strongly weathered in its upper part and separated by a nonconformity from the overlapping Destruction Bay and Cape Melville formations. The K-Ar dating of the Sherratt Bay Formation lavas is in progress.

The Destruction Bay Formation consists of reworked basaltic material, large-scale cross-bedded tuffs and psammities, occasionally with horizons rich in marine invertebrates. Fossils include *in situ* bivalves (abundant), gastropods, solitary corals and brachiopods, and with ?recycled belemnites. The channeling and megaripple character of the cross-stratified units are indicative of a near-shore marine environment. Rare conglomerate intercalations contain pebbles (not ice-raftered) of Antarctic continent provenance. These sediments formed in non-glacial conditions, as indicated by the presence of pieces of fossilized

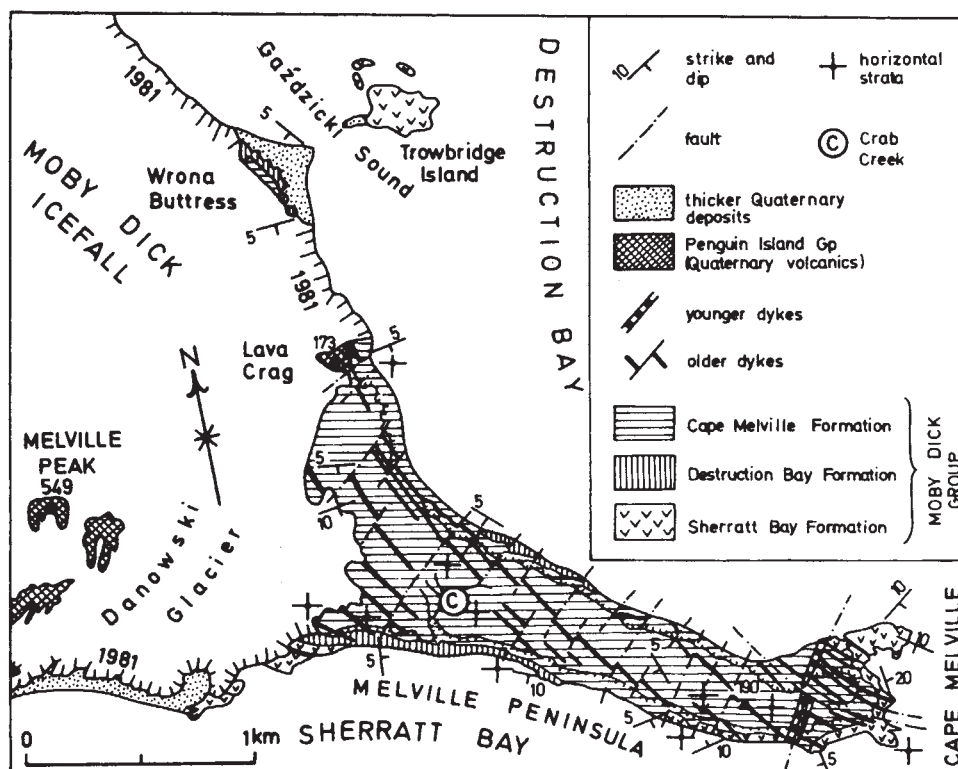
wood, and by the absence of ice-raftered dropstones. The formation is between 40 and 110 m thick and wedges out completely towards the east.

The Cape Melville Formation, ~200 m thick, consists of grey-to-green, brownish and black shale and silty shale, with subordinate siltstone and fine-grained sandstone intercalations. The formation is separated by a disconformity and basal conglomerate from the underlying Destruction Bay Formation and directly overlaps weathered basalts of the Sherratt Bay Formation on the east. Thin marl intercalations often occur in higher parts of the succession. The common occurrence of often angular, glacially-striated erratic blocks up to ~2 m in diameter, is a characteristic feature of the Cape Melville Formation. These magmatic, metamorphic and sedimentary blocks of Antarctic continent provenance are scattered at random within the sediment and have been interpreted by Birkenmajer⁶⁻⁸ as iceberg-raftered dropstones brought from the continent during a glacial epoch called the Melville Glaciation.

The Cape Melville Formation is rich in marine fossils⁶⁻¹⁰, predominantly invertebrates. Macrofossils are represented by solitary corals of the genus *Flabellum* Lesson, 1831 (abundant, and usually occurring in in-life orientation, Fig. 4b, c); polychaetes, such as the genera *Glycera* Savigny, 1811, and *Ophryotrocha* Claperède and Metschnikov, 1869; bivalves (abundant, occurring often in in-life orientation); gastropods (common); scaphopods; crabs of the section Dromiacea de Haan, 1833 (abundant, often very well preserved, Fig. 4d, e), and crab-made burrows; echinoids and asteroids; bryozoans; belemnites (common, especially in upper part of the formation). Vertebrates are represented by fish fragments. Microfossils include calcareous and arenaceous foraminifera (that is large tests of the genus *Cyclammina* Brady, 1870, Fig. 4a), the calcareous benthic genera *Pullenia* Parker and Jones, 1862, *Uvigerina* d'Orbigny, 1826, and others; abundant diatoms, mainly of the genus *Coscinodiscus* Ehrenberg, 1838 and *Triceratium* Ehrenberg, 1841; chrysomonad cysts; silicoflagellates (for example, of the genus *Distephanus* Stöhr, 1880); and sponge spicules. The above fossils are under palaeontological investigation.

Calcareous nannoplankton⁹⁻¹¹ of the Cape Melville Formation include poorly preserved Cretaceous forms: *Cribrophaerella ehrenbergi* (Arkhangelsky), *Kamptnerius magnificus*

Fig. 2 Geological map of the Cape Melville area (after refs 7, 8).



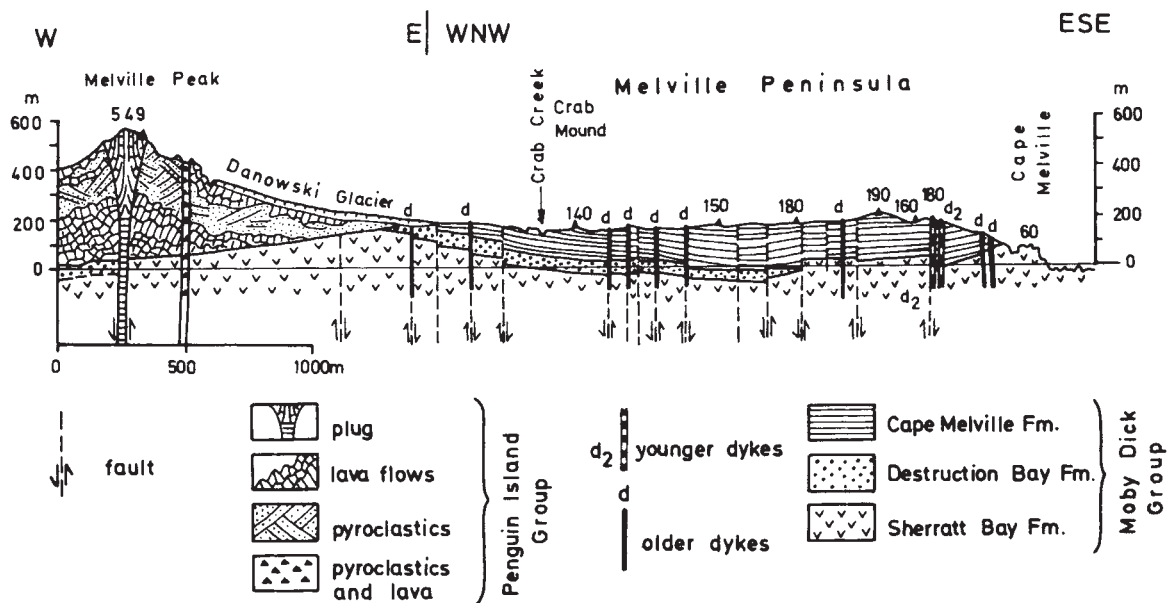


Fig. 3 Geological cross-section of the Cape Melville area (after ref. 8).

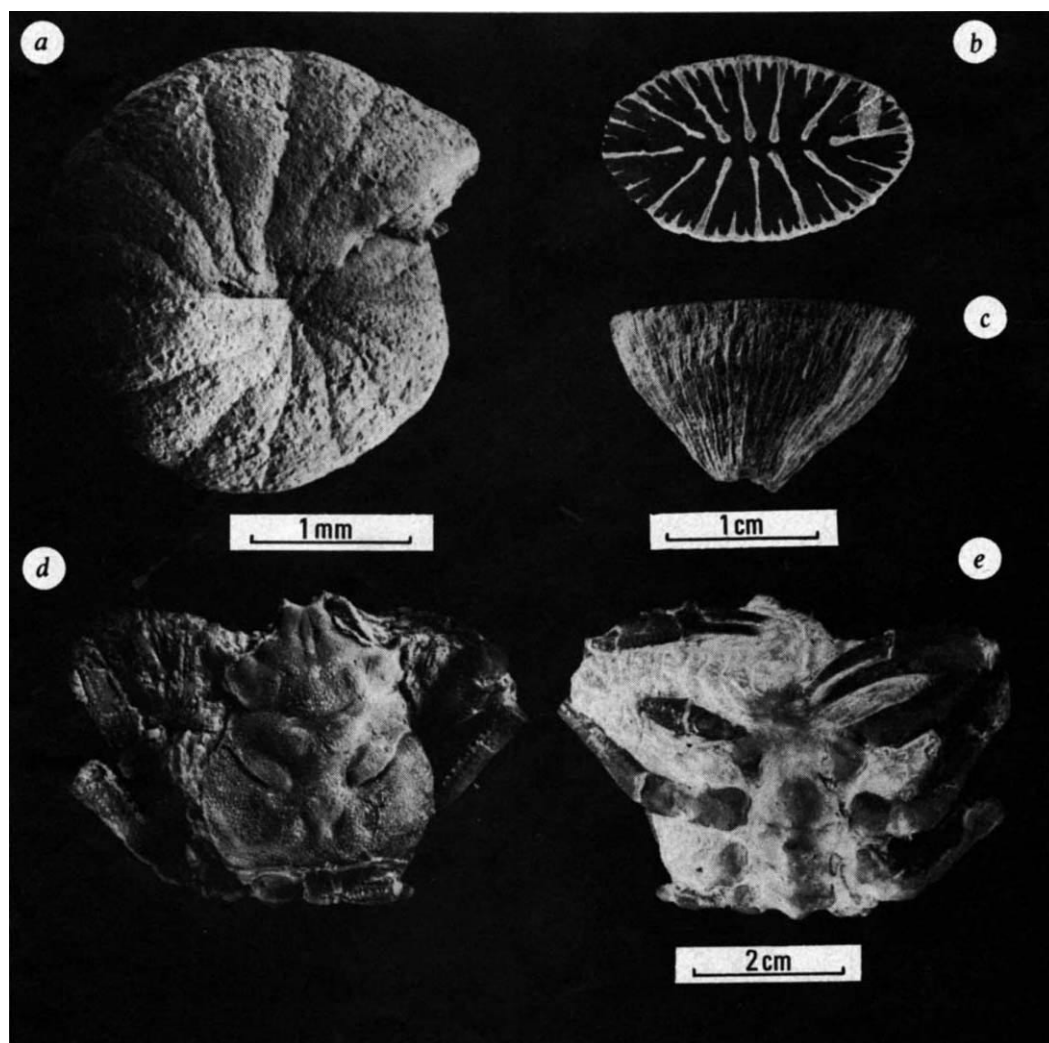


Fig. 4 Selected fauna from the Cape Melville Formation. *a*, Benthic arenaceous foraminifer of the genus *Cyclammina* Brady, 1870. *b*, *c*, Solitary coral of the genus *Flabellum* Lesson, 1831: *b*, transverse section; *c*, growth position. *d*, *e*, Crab of the section Dromiacea de Haan, 1833: *d*, dorsal view; *e*, ventral view.

Deflandre, *Discorhabdus biradiatus* (Worsley), *Marthasterites furcatus* Deflandre, *M. inconspicuus* Deflandre, *Rucinolithus irregularis* Thierstein, *Tetralithus malticus* Worsley, *T. pyramidus* Gardet, *T. obscurus* Deflandre, *T. gothicus* Deflandre, *Corollithion exiguum* Stradner, *C. achylosum* (Stover), *Hayesites albiensis* Manivit, and *Prediscosphaera cretacea* (Arkhangelsky).

The presence of Cretaceous nannoplankton together with belemnites might indicate a Cretaceous age for the Cape Melville Formation. However, there is a strong suspicion that these two fossil groups are recycled from Cretaceous strata and deposited by ice-rafting mechanisms into the Cape Melville Formation during a Tertiary glacial event. There occur some invertebrate genera, such as the foraminiferal genus *Uvigerina* (Eocene–Recent), and the coral genus *Flabellum* (Eocene–Recent) which do not appear before the Tertiary. The solution of this problem must await further palaeontological study and age evaluation of the whole fossil collection and the results of K–Ar dating of underlying basalts. At the present stage, a post-Cretaceous, and even post-lower Tertiary age of the Cape Melville Formation is suggested. It has been pointed out⁸ that there is no evidence for contemporaneous glaciation in West Antarctica as judged from the study of fossiliferous, predominantly marine sediments of the Upper Cretaceous (Campanian–Maastrichtian) Marambio Group^{12,13} and Lower Tertiary (Palaeocene–Lower Oligocene) Seymour Island Group^{12,14,15} of the James Ross Island Basin adjacent to the Atlantic margin of the Antarctic Peninsula.

The fossil assemblage of the glacio-marine Cape Melville Formation is different from that of the Pliocene glacio-marine strata of the Polonez Cove Formation^{16–18}. The field evidence suggests that the Cape Melville Formation should occur below the basalts of the Mazurek Point Formation which forms the base of glacial and glacio-marine strata of the overlying Polonez Cove Formation not far from the Cape Melville area on King George Island. A Miocene age of the Cape Melville Formation seems likely at this time⁸, considering the fact that the maximum West Antarctic glaciation occurred during the latest Miocene and early Pliocene time, with grounded ice-shelf near the Miocene–Pliocene boundary^{19–24}.

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Induced abortion and social factors in wild horses

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Much evidence now suggests that the postnatal killing of young in primates^{1–4} and carnivores^{5,6}, and induced abortions in some rodents^{7–9}, are evolved traits exerting strong selective pressures on adult male and female behaviour. Among ungulates it is perplexing that either no species have developed convergent tactics or that these behaviours are not reported, especially as ungulates have social systems similar to those of members of the above groups^{10,11}. Only in captive horses (*Equus caballus*) has infant killing been reported¹². It has been estimated that 40,000 wild horses live in remote areas of the Great Basin Desert of North America (US Department of Interior (Bureau of Land Management), unpublished report), where they occur in harems (females and young) defended by males^{13–15}. Here I present evidence that, rather than killing infants directly, invading males induce abortions in females unprotected by their resident stallions and these females are then inseminated by the new males.

Between 1979 and 1983, population regulation was examined in wild (that is, feral) horses in the Granite Range of Nevada, an insular fault-blocked mountain of ~500 km², where there are 129 horses. About 7,000 h of observation were made and the ages of over 90% of the animals are now known. Most bands vary in size from 4 to 13 and stallion tenure averages 2.11 yr (range=0.01–4.2 yr, $n=24$; for males that possessed harems when the study began, $\bar{x}=3.4$, $n=13$). Band takeovers involve aggressive fighting among males^{15,16} and forced copulations^{17–19} on unreceptive females. Of 171 copulations with harem females by resident stallions, all included aspects of male courtship (smelling, licking, nibbling and/or whinnying). In contrast, non-familiar adult females were forced to copulate 39% (14 of 36 observations) of the time by new males after persistent and aggressive biting and chasing ($z=10.62$, $P<0.001$).

To assess whether females in bands taken over by new males were less likely to reproduce than those in bands not experiencing stallion changes (that is, stable bands), the frequencies of foals borne by females were compared in bands having new and resident stallions. Foal production by females in stable bands (82%; 72 foals born of 88 possible) exceeded that by females in unstable ones (38%; 9/24). These results, however, could be confounded by female age as this influences reproduction²⁰. Thus, age effects were removed by comparing foal production in young (≤ 4 yr) and older females in stable and unstable bands. Regardless of age, females from unstable bands (old=63% foal production, young=25%) had significantly lower reproductive success than those from stable bands (old=89%, young=64%; $z=2.02$, $P<0.05$, $n=71$, and $z=2.44$, $P<0.01$, $n=41$, respectively). Thus, events associated with band instability and male takeovers mediated decreased female fecundity.

If males that acquire new females enhance their reproductive success they should increase their own genetic representation, diminish that of others, or both^{21–23}. To evaluate this prediction, I determined pregnancy and paternity rates. (1) Copulations with ejaculations were observed. I assumed the median one observed impregnated the female, which undoubtedly yielded slightly unrealistic gestation periods. Nevertheless, periods of receptivity in oestrous females are short²⁴ and better field methods are impractical. (2) Pregnancies were determined. Females were assumed pregnant in conjunction with the above

Table 1 Status of females during male takeovers and subsequent fate of their previous and new fetuses

			Month of pregnancy when encountering male				
			5th or less		6th or more		
	Maternal age (yr)	Forced copulation	Fate of new fetus		Maternal age (yr)	Forced copulation	
Previous fetus died	4	+	Aborted near birth				
	4	+	Died postnatally				
	5	+	Aborted near birth				
	6+	+	Aborted near birth				
	7+	+	Survived				
	7+	—*	Survived				
	10	—*	Survived				
	10	+	Survived				
Previous fetus lived	20	+	Survived				
					2	+	Fetus survived
					4	?‡	Fetus survived
					7+	?‡	Fetus survived
					7+	?‡	Fetus survived
					10	?‡	Fetus survived

Previous fetuses are those from prior resident stallions whereas new fetuses are those resulting from impregnations by new stallions following takeovers.

* Not forced to copulate but female returned to oestrus at least 6 weeks after takeover and was re-inseminated by the new male.

† Unclear whether the fetus of the resident stallion survived because the forced copulation occurred 30 days after the first copulation yet no subsequent oestrous cycles were detected.

‡ Band changes occurred (one individual changing twice) but discontinuous observations precluded direct knowledge of forced copulation.

condition when no further male sexual behaviour or oestrus cycle occurred for 6 weeks. This measure is conservative and may underestimate the frequency of abortions as diestrus in horses ranges from 14 to 19 days²⁵⁻²⁷. The reliability of this method depends on the subsequent detection of oestrous cycles if conception failed during the first cycle. Based on the above conditions, I estimated that 76.3% of the mares conceived during their first oestrous cycle. Of 38 copulations that resulted in parturition, 29 were during the first cycle and 9 during the next. (3) Gestation periods were calculated. As exact copulation and parturition dates were known for 38 animals, but since the season of birth affects fetal development²⁸, female gestation periods were calculated before ($\bar{x} = 337.0 \pm 8.3$ days, $n = 5$) and after ($\bar{x} = 346.6 \pm 8.1$ days; $n = 33$) 1 April. These values correspond well with those for domestic horses²⁹⁻³². Only data that fell within $2 \times \text{s.e.m.}$ (95% confidence limits) were used. Thus, of 38 oestrous cycles observed with copulations, 36 (95%) resulted in pregnancies. (4) Forced copulations and male takeovers were witnessed—that is, I made no assumptions about which males copulated or when the takeovers occurred if they were not observed.

Observations of 11 pregnant females satisfied the above four conditions. In 10 cases females were less than 6 months pregnant and following encounters with strange males, nine of the fetuses died, eight of the females were forced to copulate and, of these, 88% (7/8) of their fetuses died (Table 1). In cases ($n = 2$) where females were not forced to copulate, abortions still followed the takeovers, presumably in response to male harassment or other stresses imposed by changing social environments (see below). In 91% (10/11) of the above sample, females were re-inseminated by the new male, although not all his offspring lived (Table 1). In three of these cases, females returned to oestrus as evidenced by stallion courtship and copulation on the 8th, 14th and 16th (and again for that female on the 18th) day after forced copulation, respectively. Only one female over 5 months pregnant was forced to copulate and her fetus survived (Table 1). Four other females also changed bands when more than 6 months pregnant and they gestated successfully and produced foals at the dates predicted. This admittedly small sample suggests that most fetuses under 180 days perish *in utero* after forced copulations whereas older fetuses survive events associated with changed social environments.

The data presented above indicate that 90% of the females procured by new males when less than 6 months pregnant aborted their fetuses; 80% of these abortions were due to forced copulations. This provides strong evidence that new males increased their potential reproductive success by (1) diminishing the genetic investments of rival males, and (2) re-inseminating females that aborted (despite the 50% probability that their unborn offspring survive that year; Table 1). No cases of infanticide occurred nor did any foals disappear after male takeovers although stallions were aggressive to very young foals on three occasions.

The proximate mechanism(s) responsible for increased abortions in females that changed bands is not clear, partly due to the confounding effects of forced copulations and other stresses imposed by changing social environments. It is likely that each of these generic sets of factors contributes, particularly because females that were less than 6 months pregnant and not forced to copulate still aborted (Table 1). Although it is clear that ultimately some males enhance their reproductive success, functional considerations must still rely on which sex exerts greater control. If females were incapable of preventing abortions, the advantage would lie exclusively with males³³, which seems to be the case in the early stages of pregnancy. However, because some females failed to abort at later stages of pregnancy and others aborted despite the lack of copulations, multiple factors are certainly necessary in further considerations.

Two hypotheses concerning the evolutionary origin of induced abortion in equids can be advanced. First, it is a comparatively recent, social adaptation that received its impetus when horses were first domesticated, some time between the present and late Palaeolithic³⁴. There are no data bearing on this idea although seasonal conditions associated with post-Pleistocene periods do not support the idea of prolonged breeding seasons in Holarctic ungulates³⁵. Second, induced abortion has its evolutionary roots in related Asian and African forms; if this were the case, we would expect similarities in social organization, male aggression and birth seasonality between native equids and wild horses. Mountain zebras (*Equus zebra*)³⁶, plains zebras (*Equus burchelli*)³⁷ and Przewalski horses (*Equus przewalski*)³⁸⁻⁴⁰, all live in year-round bands, both zebra species breed throughout most of the year (as do Granite horses), and female reproductive tracts are similar among all the species⁴¹. Also, observations in zoological parks (Minnesota Zoological

Society and National Zoological Park, unpublished) have revealed forced copulations of strange Przewalski and zebra females. Thus, the possibility that induced abortion occurs in some native equids awaits confirmation from further study.

A final anomaly seems to be the disparity in frequency between induced abortion and infanticide¹² in horses. Unlike lions or primates in which the killing of neonates results in decreased inter-birth intervals¹⁻⁴, horses breed annually regardless of whether an infant lives or dies. However, the apparent paradox can be resolved by understanding the differing consequences of infanticide and induced abortion. Little would be gained by male horses that killed foals as inter-birth intervals would not be decreased and as females experience postpartum

oestrus, they would already be impregnated. Thus the alternative, induced abortion, seems a more adaptive male behaviour because it results in decreased inter-birth intervals.

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Changes in cell shape and actin distribution induced by constant electric fields

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The development of motility in cultured cells is usually associated with a polarization of the cell shape. In particular, the leading edge of the cell is extended into a lamella which acts as a locus for the elaboration of cell processes and for the formation of cell-substrate contacts and, at the opposite end, retraction fibres often extend beyond the trailing edge of the cell¹⁻³. The alignment of microfilament bundles (stress fibres) along the direction of migration and the presence of a band of actin at the leading edge of the cell suggest an involvement of this protein in the motile process⁴⁻⁶. The direction of growth and orientation of various cell types in tissue culture can be influenced by externally applied d.c. electric fields⁹ but the effect of the field on cellular motile activities is unknown. Here we describe a galvanotropic response of cultured *Xenopus* epithelial cells. At a field strength of 5 V cm⁻¹ these cells elongate perpendicularly with respect to the field. The anodal side of the cell retracts and both the ends and cathodal edge become active in the extension of ruffling lamellipodia. In parallel with the change in the cell axis, stress fibres are oriented perpendicularly to the field, and a band of actin is associated with the lamellae at the cathodal edge and at the ends of the cell.

Cells isolated from the dorsal part of *Xenopus laevis* embryos were cultured on coverslips^{10,11}, and epithelial cells were identified according to published criteria¹⁰. These cells were

extremely thin and well spread with an average width of 125 μ m (Fig. 1a). The periphery was often expanded into flattened cytoplasmic sheets, whose margins exhibited lamellipodial extensions and ruffles. Although these structures resembled those occurring at the leading edge of motile fibroblasts, they were not restricted to one pole of the cell, which therefore did not exhibit a directional elongation.

When a constant electric field of 5 V cm⁻¹ was applied to the culture, a marked reorientation of the cells occurred. After a 2-h exposure, 86% of the cells were elongated on an axis which was within 15° of perpendicular to the field (Table 1). Whereas the average sine of the angle between the longest axis of control cells and an arbitrary direction was 0.71 (corresponding to a random distribution in cell orientation), that of the field-exposed culture was 0.95 (within 5% of the theoretical limit of 1). From an analysis of the cell dimensions with respect to the field direction it was determined that this reorientation resulted from a 60% increase in cell length perpendicular to the field and a 40% decrease in cell width parallel to the field (Table 1).

To identify the cell activities involved in the development of this reorientation, selected cells were monitored by time-lapse video recording and photography before and during field exposure. In the absence of the field, the extension of cell processes often resulted in shape changes, but persistent unidirectional migration was rare during the 90-min observation period (Fig. 1a, b). Changes in cell shape usually became detectable within 15 min of field application (Fig. 1c); these were usually initiated by a contraction of cell processes extending towards both the anode and cathode, and the simultaneous or subsequent formation of ruffling lamellipodia towards the cathode or at the poles perpendicular to the field (Fig. 1e, f). Lamellipodia underwent repeated extension and retraction at the cathodal edge, which was considerably more active than the periphery of control cells. These structures commonly translocated towards one or both poles perpendicular to the field, which underwent a comparatively constant expansion. Lamellipodial ruffling and extension was not observed at the anodal edge, which generally retracted until it was relatively straight

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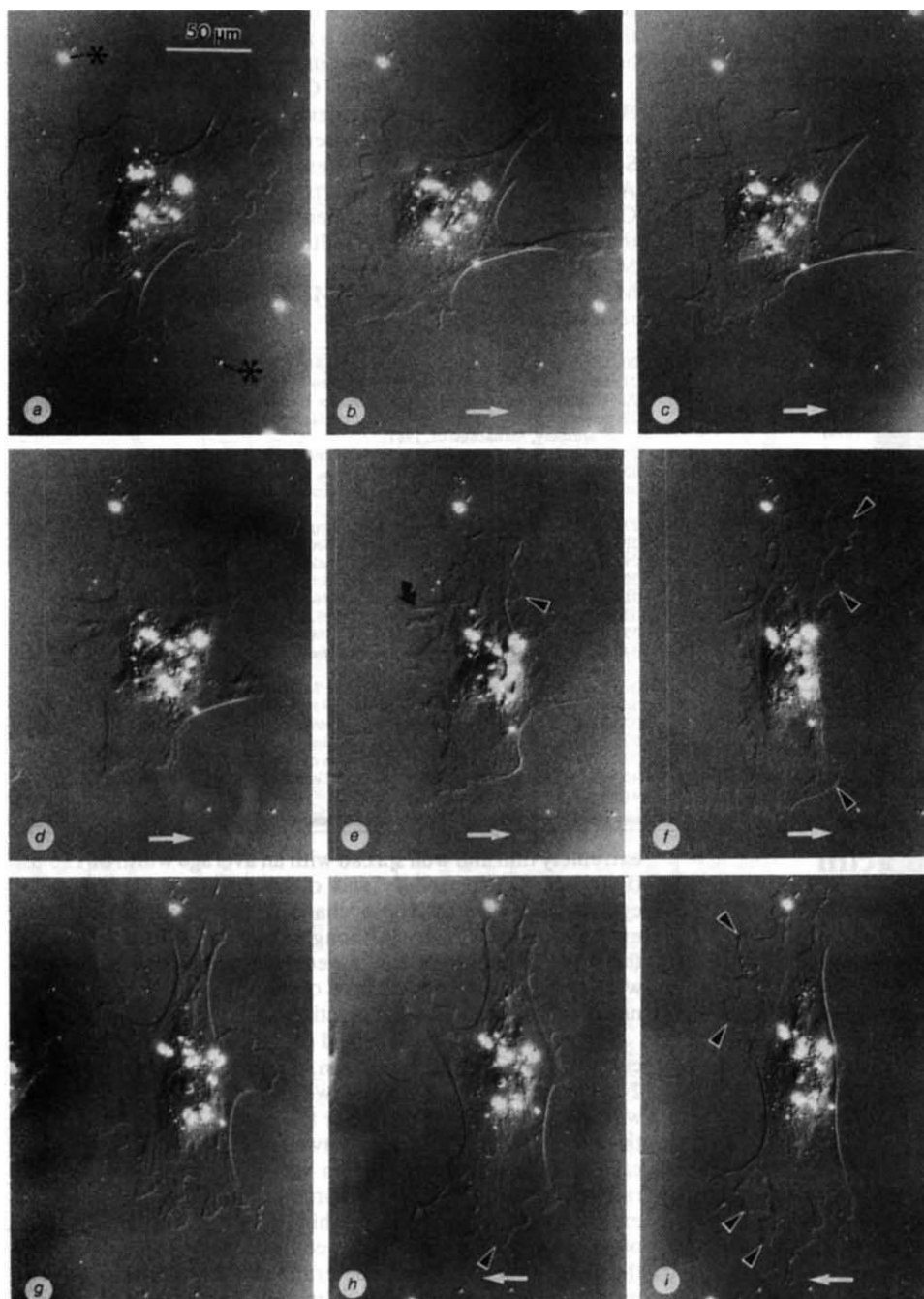


Fig. 1 Sequential micrographs of an epithelial cell monitored continuously during its exposure to a field of 5 V cm^{-1} . *a*, 90 min before application of the field; *b*, at the onset of the field; *c-f*, during field exposure at 15 (*c*), 30 (*d*), 60 (*e*) and 90 (*f*) min; *g*, 60 min after field termination; *h, i*, during the application of a reverse field at 30 (*h*) and 60 (*i*) min. White arrows point towards the cathode. Arrowheads indicate lamellipodia, which were seen along the cathodal edge and at the two ends of the cell (*e, f* and *h, i*). Curved arrow indicates retraction fibres along the anodal side of the cell. The procedure described in Table 1 was modified to allow field application during microscopic observation with interference contrast (Nomarski) optics using a $\times 25$ objective. The asterisks in *a* indicate stationary objects on the substrate.

and perpendicular to the field, leaving behind retraction fibres (Fig. 1*e*). Occasionally retraction of cathodal processes formed after the application of the field also left retraction fibres (Fig. 1*e*), indicating that these processes were able to form substrate adhesions. Despite these activities, cell migration was rarely observed at this field strength. However, a $60 \mu\text{m h}^{-1}$ sideways translocation of the extended cells towards the cathode was observed when the field strength was increased to 15 V cm^{-1} .

Termination of the field was followed by the formation of cell processes towards the original anode as well as the cathode. The edges perpendicular to the field either continued to expand or retracted. Thus the cell shape and orientation usually relaxed to an unpolarized condition within 30–60 min (Fig. 1*g*). Subsequent application of a reversed field (Fig. 1*h, i*) resulted in a similar response with respect to the new field, that is, retraction of the new anodal edge, increased lamellipodial ruffling and extension at the new cathodal edge, and an eventual orientation of the cell perpendicular to the field.

Indirect immunofluorescence was performed to determine the relationship between these field-induced changes and the

distribution of actin. In controls, stress fibre distributions ranged from paralleling the cell axis (Fig. 2*a, b*) to a radial disposition (Fig. 2*c, d*). A band of actin was often located at the periphery of the cell (Fig. 2*a, c*). Stress fibres in cells exposed to electric fields displayed a striking orientation perpendicular to the field, and commonly traversed the entire length of the cell (Fig. 2*e-h*). Peripheral actin bands were located at the perpendicular and cathode-facing lamellae (Fig. 2*e, g*), and coincided with dark bands in the phase-contrast image (Fig. 2*f*). These bands were absent from the anodal edge, where the cell cortex was closely associated with stress fibres (Fig. 2*e, g*).

These results indicate that the electric field can polarize a set of motility-related cell activities and the distribution of actin structures in cultured *Xenopus* epithelial cells. This polarization includes a correlation of the orientation of cell elongation and stress fibres, and of the localization of lamellipodial expansion and peripheral actin bands. The latter closely resembles the arrangement at the leading edge of motile fibroblasts, but differs in that cell expansion towards the cathode commonly occurs perpendicular to stress fibres and the long axis of the cell. Thus,

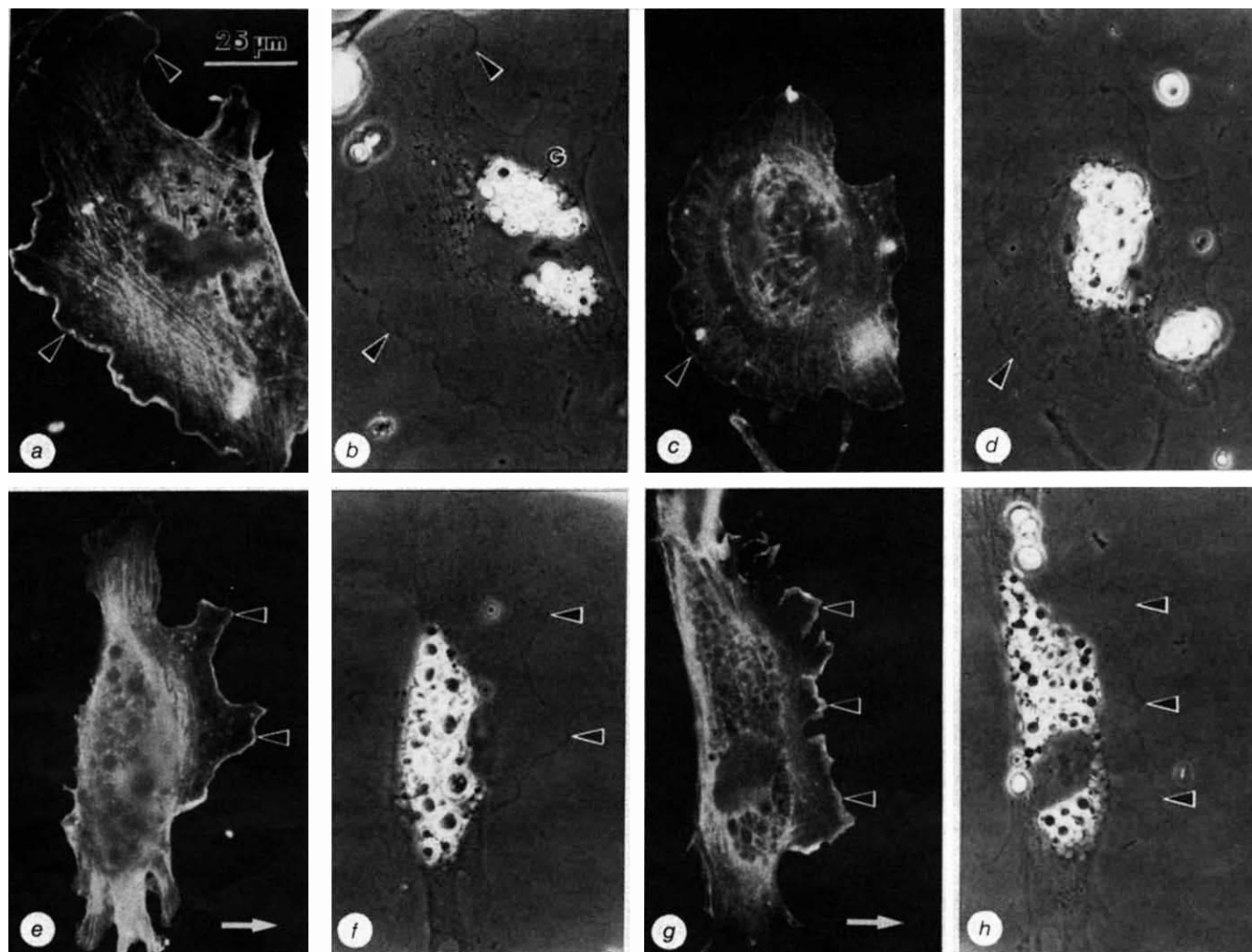


Fig. 2 Distribution of actin in control and field-exposed epithelial cells. *a, c, e, g*, Fluorescence micrographs; *b, d, f, h*, the corresponding phase-contrast images. The cultures were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 10 min, permeabilized with 95% ethanol at -20°C and reacted with a mouse monoclonal anti-actin antibody (JLA20)²⁸ followed by labelling with fluorescein isothiocyanate-conjugated goat anti-mouse IgG. Observation was carried out with a Leitz Orthoplan microscope using a $\times 63$ objective (N.A. 1.4). *a-d*, Cells from control cultures. In addition to stress fibres, a band of actin was associated with the periphery of the cell (arrowheads in *a, c*) which coincides with a phase-dark band in these cells (corresponding arrowheads in *b, d*). G, yolk granules. *e-h*, Cells from cultures exposed to a 5 V cm^{-1} field for 4 h. Arrows indicate the direction of the cathode. The stress fibres became oriented perpendicular to the field and the actin bands were localized in lamellipodia at the cathodal edge of the cell (arrowheads).

Table 1 Effects of electric fields on epithelial cell shape and orientation

	Maximum length, l^* (μm)	Maximum width, $w^\dagger$ (μm)	Ratio (l/w)	Average sine	Deviation from perpendicular ‡ (degrees)	Perpendicular extent § (μm) (<i>a</i>)	Parallel extent $^\parallel$ (μm) (<i>b</i>)	Ratio (<i>a/b</i>)
Control	140.9 ± 5.0	75.5 ± 4.0	1.9	0.71 ± 0.04	40.7 ± 3.9	119.3 ± 4.5	112.5 ± 4.8	1.1
Field-exposed	195.0 ± 9.2	52.7 ± 2.9	3.7	0.95 ± 0.02	10.9 ± 2.6	191.9 ± 9.6	69.7 ± 2.8	2.8

Cells were isolated from the dorsal half of Nieukoop and Faber²⁷ stage 22 *X. laevis* embryos and cultured on no. 1 coverslips in Steinberg's solution (60 mM NaCl, 0.7 mM KCl, 0.4 mM $\text{Ca}(\text{NO}_3)_2$, 0.8 mM MgSO_4 , 10 mM HEPES, pH 7.4), supplemented with 10% L-15 (Leibovitz) medium and 1% fetal bovine serum^{10,11}. For the application of the electric field, the culture was inverted onto a $0.2 \times 10 \times 62$ mm chamber whose bottom was made of a glass slide and whose walls were made of no. 2 coverslips. The chamber was filled with culture medium. The current was applied to the chamber via a pair of Ag-AgCl electrodes and two sets of 1% agar bridges prepared with Steinberg's solution, which separated the culture from the electrodes. A 5 V cm^{-1} field (calculated from the applied current density and the medium conductivity) was applied for 2 h then the culture was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. Observations were made with a $\times 40$ planapochromatic phase-contrast objective on a Leitz Orthoplan microscope with a RCA TC1005N video camera. Measurements were made directly on the monitor screen. Data are the mean $\pm$ s.e.m. for 35 randomly selected cells. For the control cultures not exposed to the field, the 'direction of the field' was designated arbitrarily. Average sine = $\sum_{i=1}^n \sin \theta_i / n$, where θ_i is the angle between the long axis of the cell and the direction of the field; $n = 35$ (total number of cells scored). The cathode and anode were at 0° and 180° , respectively.

* Maximum length of the cell, excluding processes $< 3\text{ }\mu\text{m}$ wide.

† Maximum width of the cell perpendicular to l , excluding processes $< 3\text{ }\mu\text{m}$ wide.

‡ The deviation of the angle of the long axis of the cell from perpendicular to the field.

§ The vertical distance between the perpendicular extremes of the cell with respect to the field, excluding processes $< 3\text{ }\mu\text{m}$ wide.

$^\parallel$ The horizontal distance between the anodal and cathodal extremes of the cell with respect to the field, excluding processes $< 3\text{ }\mu\text{m}$ wide.

our data indicate that lamellipodial extension is independent of stress fibres (see also ref. 12). On the other hand, the peripheral actin band in these structures may be closely related with cell motility. This is consistent with evidence that the motile areas of migrating cells are usually associated with a diffuse distribution of actin⁷. The failure of the cells to move despite cathodal extension may be due to the presence of focal contacts at the ends of the stress fibres^{2,8,13}; it seems that this restriction is overcome at higher field strengths. Several other galvanotropic effects bear a close relationship to our results. Neurites in tissue culture undergo a preferred elongation towards the cathode of applied d.c. fields¹⁴⁻¹⁷. The elongation of the neurite at the growth cone may be analogous to the cell expansion along the cathodal edge described above; despite the absence of stress fibres, a meshwork of F-actin has been observed at the growth cone¹⁸, which may resemble the actin band along the cathodal edge reported here. On the other hand, both cultured 3T3 fibroblasts (P.W.L., H.B.P. and R. J. Walter, unpublished results) and spreading *Xenopus* myotomal cells¹⁷ have stress fibres, and both become oriented perpendicular to applied fields. However, once the muscle cells spread out, they fail to be reoriented by the field (P.W.L. and H.B.P., unpublished results). These results suggest that the ability of cells to change orientation may be related to the flexibility of stress fibres to undergo reorientation. Myofibrils can be regarded as a highly differentiated form of stress fibre¹⁹ and are integrated by a lattice of intermediate filaments²⁰. Therefore spread *Xenopus* myotomal cells, which acquire myofibrillar differentiation within the first day of culture¹⁹, would soon become resistant to reorientation. On the other hand, constant electric fields of the magnitude used here are known to cause an aggregation of acetylcholine and concanavalin A receptors towards the cathode in these *Xenopus* muscle cells^{21,22}. As the redistribution of surface receptors may involve membrane-associated actin filaments²³, it is possible that a cathodal band of actin similar to that described here may also have a role in the field-induced receptor aggregation in muscle cells.

Endogenous d.c. electric currents exist in a variety of plant and animal tissues⁹. However, whether these natural currents can generate electric fields of sufficient magnitude to direct cell motility *in situ* remains to be demonstrated. Nevertheless, our results show that d.c. electric fields can be used as a simple experimental means of elucidating the involvement of the cytoskeleton in cell motility. After this paper was prepared, three other reports concerning the field-induced orientation of cells appeared in abstract form²⁴⁻²⁶.

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Bathocuproine sulphonate: a tissue culture-compatible indicator of copper-mediated toxicity

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Metal-binding chelators may interact with biological systems by either of two mechanisms: they may combine with an essential metal, which can be either freely dissociated or part of an enzyme prosthetic group, or they may react with a metal ion to form a biologically reactive metal-chelate complex¹. As trace metals are always present as contaminants in serum-supplemented culture media used to study chelating agents, it is frequently difficult to distinguish between the two possibilities. Here we describe the use of a nontoxic, copper-specific chelating agent, bathocuproine sulphonate^{2,3} (Fig. 1) which, by combining with available endogenous copper in a tissue culture preparation, abolished the toxicity of three structurally unrelated chelating agents. These three agents may therefore be considered to be biologically active by the second mechanism.

Metal chelates of diethyldithiocarbamate (DDC) have long been recognized as fungicidal, bacteriocidal^{4,5} and radiosensitizing agents⁶. However, DDC has more recently been used for its property of inactivating superoxide dismutase, both *in vivo*⁷ and in tissue culture⁸, by removing essential copper (or zinc) from this enzyme. Its biological action was therefore presumed to be due to the inhibition of this enzyme, and subsequent cell damage was attributed to oxygen radicals which would normally be inactivated.

We have examined the effect of micromolar concentrations of DDC on the growth of L1210 murine leukaemia cells in culture. Figure 2A shows that it was toxic to the cells and at increasing concentrations produced a limiting inhibition of growth of 80%. Added copper potentiated the inhibition and added bathocuproine sulphonate (BCS) abolished it. Copper alone, at concentrations up to 100 μ M, did not inhibit cell growth. These observations indicate that the toxicity of DDC in tissue culture is not due to inhibition of superoxide dismutase because such inhibition would be blocked, not potentiated, by addition of copper.

Many reports have confirmed that 2-keto-3-oxobutylaldehyde bis(thiosemicarbazone)—KTS—requires divalent copper for both its antitumour activity as well as for cytotoxicity in culture^{9,10}. Nanomolar concentrations of KTS were toxic to L1210 cells (Fig. 2B) and again, added copper potentiated, and BCS abolished, this growth inhibition.

The results obtained with 2,9-dimethyl-1,10-phenanthroline (2,9-DMP) were most striking (Fig. 2C). This compound has the same ring system and location of methyl groups as BCS but lacks the sulphonated phenyl moieties. As with DDC and KTS, 2,9-DMP produced a limiting toxicity at increasing concentration, and this toxicity was potentiated by copper and alleviated by BCS. This is remarkable as 1,10-phenanthroline,

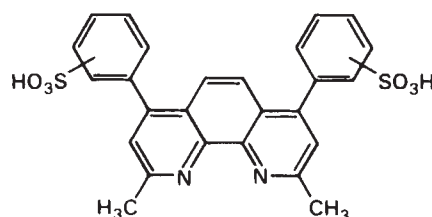
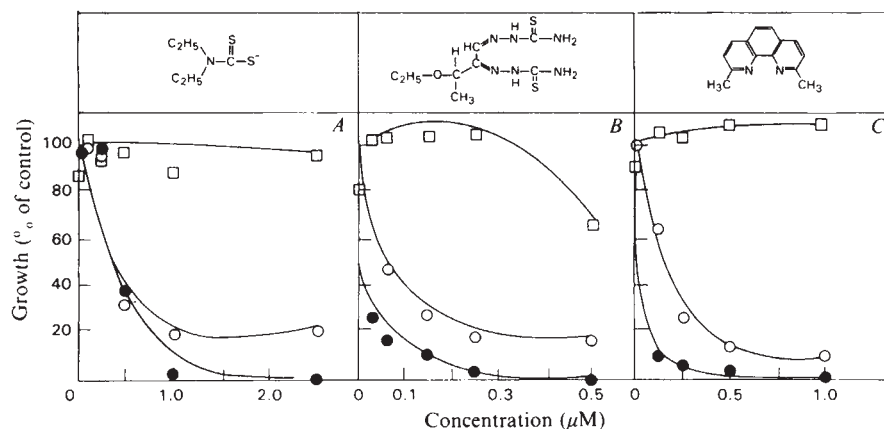


Fig. 1 2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline disulphonic acid; bathocuproine disulphonic acid.

Fig. 2 Effect of chelating agents on growth of L1210 cells in culture. The effect of diethyldithiocarbamate (A), 3-ethoxy-2-oxobutylaldehyde bis(thiosemicarbazone) (B) or 2,9-dimethyl-1,10-phenanthroline (C) on cell growth is shown either alone (○), with 25 μ M copper sulphate (●), or with 25 μ M bathocuproine sulphonate (□).

Methods: The L1210 established culture line was maintained in RPMI 1630 growth medium (K. C. Biologicals, Lenexa, Kansas) containing 16.5% fetal calf serum (Flow). Cells were collected at mid-log phase ($8-10 \times 10^5$ cells ml^{-1}), washed three times with medium containing 40 $\mu\text{g ml}^{-1}$ gentamicin (Schering) and adjusted to 1×10^5 cells ml^{-1} . Diethyldithiocarbamate (Fisher Scientific), 3-ethoxy-2-oxobutylaldehyde bis(thiosemicarbazone) (Research Organics) and 2,9-dimethyl-1,10-phenanthroline (G. Frederick Smith Chemical Co., Columbus) were dissolved in water, dimethylformamide and ethanol, respectively. (These solvents were nontoxic at the concentrations used.) Bathocuproine sulphonate (G. Frederick Smith Chemical Co.) and copper sulphate were dissolved in water. The effects of these compounds on cell growth were determined from the growth fraction ($N/N_0 - 1$) calculated by using a Coulter Counter, model ZBI, after 45 h at 37 °C.



the parent ring system compound, is toxic to cells in culture but becomes nontoxic on the addition of copper, zinc or iron^{11,12}. This specificity in the toxicity of the 2,9-dimethyl derivative may be due to the unique position of the methyl groups proximate to the metal coordination site, a change which introduces steric hindrance on coordination and results in an increased redox potential^{13,14}. Indeed, it may be the high redox potential resulting from steric hindrance in the planar cupric bidentate chelate which makes BCS such a good copper scavenger, for this would facilitate the reduction of the less stable cupric chelate to the stable, tetrahedral cuprous chelate by biological reductants in the medium.

L-2-Amino-3-(hydroxynitrosamino)propionic acid, commonly known as L-alanosine, is an antibiotic with potential antitumour use¹⁵, which binds copper tightly¹⁶. A recent report has indicated no significant difference in its antitumour activity when animals were copper-deficient or copper-loaded¹⁶. When tested against L1210 cells in culture, the cytotoxicity of L-alanosine was unaffected by adding copper or BCS (data not shown), confirming the *in vivo* work cited above.

Thus, BCS, when tested against four chelating agents, has proved to be a reliable indicator of copper-mediated toxicity. The results obtained confirm the previously known copper-dependence of both DDC and KTS, as well as the non-dependence on copper of the cytotoxicity of L-alanosine, and demonstrate that the 2,9-DMP copper complex is a potent cytotoxin to L1210 cells in culture. We conclude that DDC, KTS and 2,9-DMP are therefore not cytotoxic by virtue of their ability to combine with essential metals on enzymes, and that L-alanosine is cytotoxic by a mechanism independent of copper. The lack of toxicity of BCS in culture and its specificity for copper makes it a potential therapeutic agent for alleviation of copper toxicity *in vivo*.

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Inhibition of axonal growth by a monoclonal antibody

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Little is known about factors which control the outgrowth of neurites in the central nervous system (CNS) during development, although *in vitro* studies with neurones from the peripheral nervous system have shown that neurites require an appropriate substrate¹ and specific factors^{2,3} for growth. To investigate the role of cell-surface components in the development of the CNS, we have raised a series of monoclonal antibodies against membrane components of cells from the visual system of the chick embryo. We report here that one of these antibodies, T61/3/12, clearly inhibits axonal outgrowth from chick retinal explants but does not affect axon growth from the peripheral nervous system.

Membranes were prepared from retinæ of 9-day-old chick embryos as described by Halfter *et al.*⁴. 100 μg of membrane protein were injected 20 times at weekly intervals intraperitoneally and intravenously into female BALB/c mice; 3½ days after the last boost, spleen cells from one mouse were fused with P3-NS1/1-Ag4-1 myeloma cells (given by Dr C. Milstein) by the procedure described by Saumweber *et al.*⁵. Cells were plated out into microtitre plates at 6×10^5 cells per well in hypoxanthine-aminopterin-thymidine medium on layers of peritoneal macrophages⁶. Hybridoma clones were screened for antibody production by indirect immunofluorescence on 9-day-old chick embryo retina cells grown for 1 day on concanavalin A coated coverslips⁷. Hybrids producing antibodies that bound to the surface of living retina cells were selected and cloned twice by limiting dilution; 25% of the cultures were positive according to these criteria.

One stable cell line, T61/3/12, was selected for the study described here. It produces an antibody which shows remarkably high binding to the neurite-containing plexiform layers of the embryonic chick retina (Fig. 1) in 4% paraformaldehyde-fixed frozen sections. During embryonic days 5-17, most labelling is confined to the axon layer and the developing plexiform layers, whereas much weaker ring-like staining can be seen over the nuclear layers (Fig. 1a-d). Retina sections from adult animals show almost the same staining pattern as day 17 retinæ. Figure 1f shows intense staining of plexiform layers is not only due to their higher membrane content. The

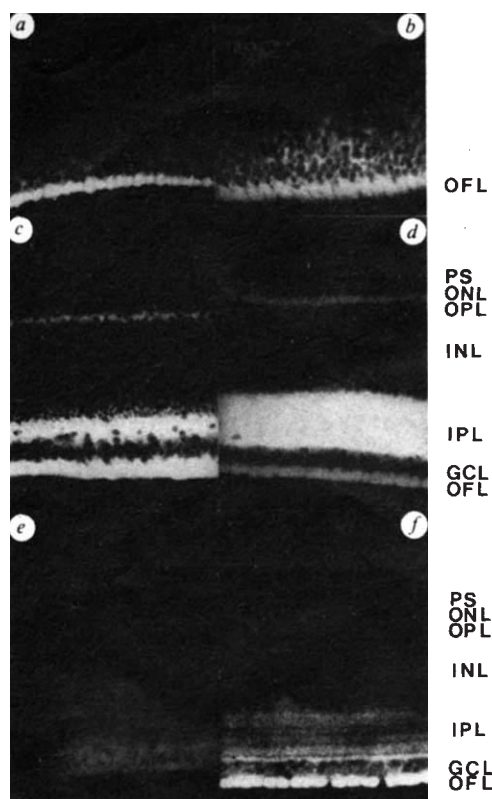


Fig. 1 Localization of T61/3/12 binding in embryonic chick retinae of various ages. *a*, 5-day retina, T61; *b*, 7-day retina, T61; *c*, 9-day retina, T61; *d*, 17-day retina, T61. *e*, Shows a control with culture medium; *f*, the staining with a different antibody, W 102, which was applied undiluted as cell culture supernatant on a 17-day retina. PS, photoreceptor segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFL, optic fibre layer. Retinae from 5-, 7- and 9-day-old embryos were isolated intact and spread on black membrane filters (SM 13006; Sartorius). Pieces from the central part were cut out and fixed for 6 h at 4 °C in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. After fixation the retinae were soaked in 30% sucrose (w/v) overnight. Eyes from 17-day-old embryos were fixed intact by injecting 4% paraformaldehyde into the vitreous body and then immersing the eye in the fixative. The eyes were soaked in sucrose as described above. The retina was then spread on a filter and a piece from the more nasal part was cut out. Retinae were frozen in isopentane at -180 °C. Sections (10- μ m) were cut on a cryostat at -20 °C and dried onto gelatine-coated slides. Sections were preincubated with 10% normal rabbit serum in phosphate buffer for 10 min at room temperature and then with T61/3/12-ascites (1:30 diluted with phosphate buffer) for 18 h at 4 °C. The slides were washed in two changes of a large excess of phosphate buffer for 15 min each. Bound immunoglobulin was detected by further incubation for 30 min at room temperature with fluorescein-conjugated rabbit anti-mouse immunoglobulin (Miles, diluted 1:40 in phosphate buffer). The slides were again washed with three changes of phosphate buffer and then a coverslip was applied using 1 mg ml⁻¹ *p*-phenylenediamine in phosphate buffer as mounting medium. Slides were examined by phase-contrast and epifluorescent illumination on a Zeiss microscope. Photographs were taken with Kodak Ektachrome colour slide film (400 ASA). Exposure times were 5-10 s for immunofluorescence.

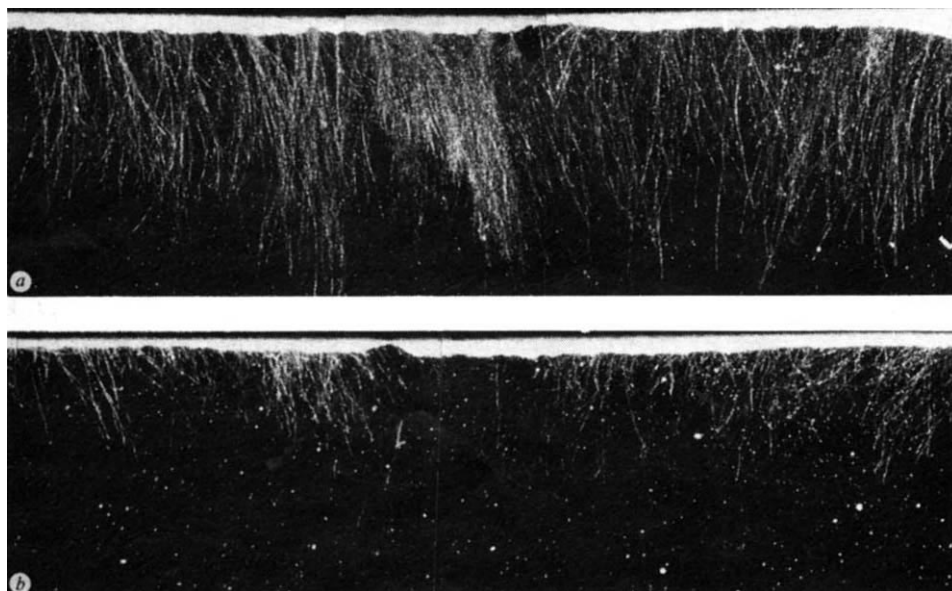


Fig. 2 Inhibition of axonal outgrowth from chick retinal explants by T61/3/12 antibody. A retina from a 6-day-old chick embryo was isolated intact and spread on a black membrane filter (SM 13006; Sartorius)⁴, 0.275-mm strips—extending from the nasal to the temporal part—were cut and inverted onto collagen-coated Petriperm dishes, the ganglion cell layer facing the collagen. Alternate strips from one retina were placed into two dishes. After 2 h at 37 °C, 1.3 ml culture medium⁴ (control) or the same amount of culture medium, containing 30 μ g T61/3/12 antibody per ml, were added. The explants were incubated at 37 °C and 4% CO₂ for 40 h. They were fixed with 1% paraformaldehyde and observed by dark-field illumination. Formerly neighbouring strips were compared and photographs were taken with Ilford Pan F film. *a*, Control explant; *b*, explant with added T61/3/12 antibody.

antibody W102 used for incubation of this section seems to stain all levels of the retina equally, with the exception of the optic fibre layer. As shown by immunofluorescence of explants and in retinal cell cultures, T61/3/12 antibody binds to axons, growth cones and perikarya. Labelling has also been seen on cultured cells from the optic tectum and cerebellum but not on fibroblasts or heart muscle cells.

Because of the high level of antibody binding to neurites, we tested T61/3/12 hybridoma culture supernatants for the influence they might exert on outgrowing axons. We used the *in vitro* system described by Halfter *et al.*⁴ in which strips from 6-day-old embryonic chick retinae are explanted on collagen-coated Petriperm dishes. Vigorous outgrowth of fibres, which could be shown to be exclusively ganglion cell axons⁸, was substantially reduced by adding T61/3/12-containing super-

natant. Several other hybridoma supernatants that showed binding to retinal fibres in indirect immunofluorescence tests did not affect axonal growth.

Figure 2 shows the inhibitory effect of T61/3/12 antibody at a concentration of 30 μ g per ml. Explants from one retina—0.275-mm strips extending from the nasal to the temporal part of the retina of a 6-day-old chick embryo—were placed in two collagen-coated Petriperm dishes. Two hours later, 1.5 ml culture medium⁴ (control, Fig. 2*a*) or culture medium mixed with immune ascites from T61/3/12 tumour-bearing mice (Fig. 2*b*) was added. Comparison of two formerly neighbouring retinal strips over a period of 40 h in culture shows that the axons are highly reduced in number and length after incubation with T61/3/12 antibody. Lower antibody concentrations (5 μ g ml⁻¹) are equally effective. Neighbouring strips were chosen because

in control experiments they show minimal variation in length of neurite outgrowth. Fourteen other ascites fluids containing different antibodies of comparable concentrations did not influence outgrowth of retinal axons when tested in this system.

Time lapse recordings of retinal axons growing over either a monolayer of retinal cells⁷, or a collagen or laminine-coated surface revealed that immediately on addition of T61/3/12 antibody most of the growth cones stopped their searching movements, and remained firmly attached to the substrate. As inhibition of growth was observed on several substrates, it cannot easily be explained by cross-linking of the growth cones to the substrate by T61/3/12. Further work with Fab fragments will help to exclude cross-linking phenomena. Filopodia were still visible and at least some of them were actively moving in the medium. None of these axons could be seen to grow again during the observation period of 5 h after addition of the antibody. A minority seemed to be unaffected and continued to move. We do not know whether these axons represent neurites from a different ganglion cell class. Inhibition of neurite outgrowth was reversible, because axons started to regrow about 1 h after thorough washing of the explants with culture medium.

Dorsal root ganglia, explanted from 9-day-old chick embryos and cultured for 3 days in the presence of T61/3/12 antibody (T61/3/12-ascites, 1:20 in Dulbecco's modified Eagle's medium + 10% heat-inactivated fetal calf serum + 20 ng ml⁻¹ β -nerve growth factor) on polylysine-coated microtitre plates or on laminine-coated coverslips were not inhibited in their axonal outgrowth by the antibody. The axons reached the same length as in cultures without antibody or with the addition of a control ascites fluid (antibody P12, directed against chromosomal proteins of *Drosophila melanogaster* (ref. 5); given by P. Symmons). This result was confirmed by cinematographic studies. The growth cones of dorsal root ganglion axons that had grown *in vitro* for 1–3 days were unaffected by the addition of T61/3/12. Also, the indirect immunofluorescence test showed that they do not bind the antibody.

The effect of T61/3/12 antibody does not seem to be due to general cytotoxicity. In monolayer cultures of 9-day-old retinal cells, where ~40% of the cells express the antigen, no dead cells could be observed after brief or overnight incubation with the antibody, as judged by trypan blue exclusion. Furthermore, the very fast inhibition of growth cone movement suggests a specific mode of action.

A preliminary investigation of the species distribution of the antigen revealed that retinal cells from 2-day-old postnatal mice as well as frozen sections from adult mouse retina show no antibody binding. Retinal cells from 8-day-old quail embryos are stained with the same intensity as chick cells.

The immunoglobulin class of the antibody has been shown by immunoprecipitation and immunofluorescence with class-specific antibodies to be IgG3, but the chemical nature of the antigen has not been determined. It seems that the molecule is relatively insensitive to mild digestion by a 0.1% solution of trypsin or papain. However, on incubation with 0.1% pronase, antibody binding is reduced to ~50% (R. Babel, personal communication), suggesting that the antigen might be a protein or glycoprotein. Fixation of cells with 96% ethanol does not abolish antibody binding capacity and thus the antigen does not seem to be a ganglioside.

Previous studies have identified several molecules involved in cell aggregation in the chick retina. The T61/3/12 antigen, although not yet identified, can be distinguished from the cell adhesion molecule (CAM)⁹ by its stability to trypsin and its absence in mouse retina¹⁰ and chick dorsal root ganglia¹¹. The antigen differs from cognin¹² in being present on freshly trypsinized retinal cells and by its expression on central nervous system tissue other than the retina. The strong binding of T61/3/12 antibody to plexiform layers shows some resemblance to the properties of RET5 antibody described by Lemmon and Gottlieb¹³. In contrast to T61/3/12, however, this antibody does not stain cell perikarya.

The apparently specific inhibitory action of the T61/3/12 antibody on outgrowth *in vitro* of chick retinal ganglion cell axons suggests that the antibody is directed against a molecule that might either have an important role in the interaction between growth cone and substrate or may serve as a receptor for growth factors that stimulate neurite outgrowth. Characterization of the chemical nature of the antigen (work in progress) may help to determine its mode of action.

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Genetic dissection of monoamine neurotransmitter synthesis in *Drosophila*

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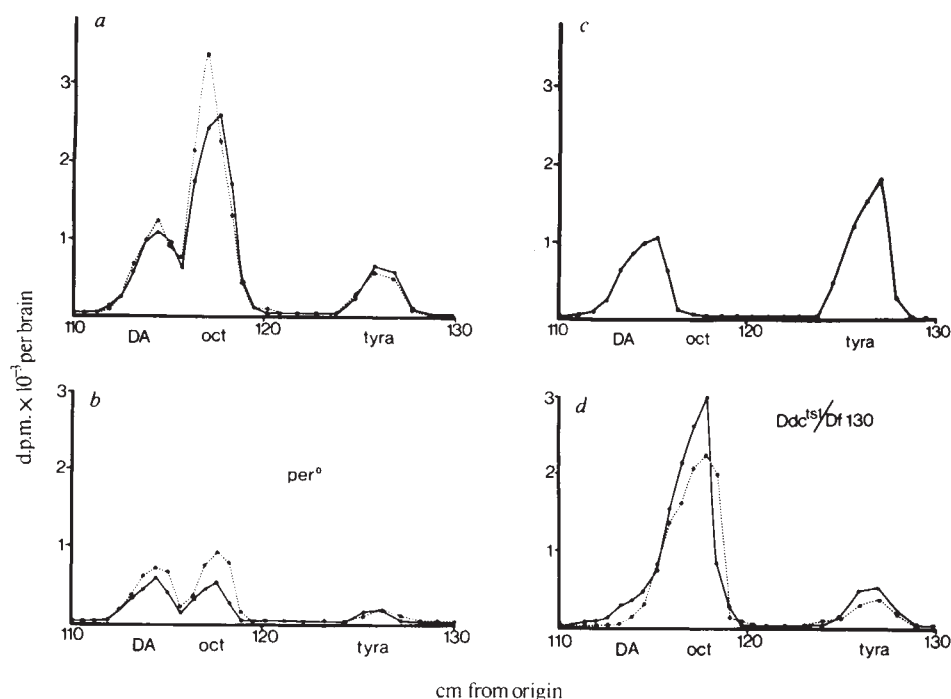
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The biogenic monoamine neurotransmitters octopamine, dopamine and serotonin have been detected in nervous tissue from many insects¹. We report here that intact *Drosophila melanogaster* brains, when incubated with the radioactive amino acids tyrosine and tryptophan, synthesized and accumulated labelled monoamines. In two mutant strains monoamine synthesis was abnormal. The *per*⁺ mutation abolishes the normal circadian rhythm^{2–4}. Brains from *per*⁺ flies, when incubated in tritiated tyrosine, accumulated one-third as much labelled octopamine as did brains from wild-type flies, but had normal dopamine and serotonin synthesis. In contrast, dopa decarboxylase (*Ddc*) mutations^{5,6} decreased dopamine and serotonin synthesis but did not affect octopamine synthesis. These results suggest that there are two different aromatic amino acid decarboxylases in *Drosophila* brains, one that decarboxylates L-dopa and 5-hydroxytryptophan and another that decarboxylates tyrosine. Direct measurement of L-dopa, 5-hydroxytryptophan and tyrosine decarboxylase activities in the different strains confirmed this suggestion.

The study of monoamine neurotransmitters in flies is complicated by the fact that dopamine and possibly other monoamines are important intermediates in cuticle tanning^{7–9}, and consequently have a high rate of synthesis and metabolism in the epidermis¹⁰. To study monoamines in their role as neurotransmitters we developed a technique in which brains were dissected intact from either adult or larval *Drosophila*, and incubated in a small hanging drop with radioactive precursors to measure monoamine synthesis and accumulation. The brains were incubated for 30–60 min in oxygenated saline containing tritiated tyrosine or tryptophan and the radioactive products were separated by high-voltage electrophoresis. The major metabolites

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Fig. 1 High-voltage electrophoresis profile of *Drosophila* brains incubated in ^3H -tyrosine. Dotted and solid lines represent duplicate experiments. Eight intact adult brains with cuticle removed were incubated for 30 min at 25 °C in a 10- μl hanging drop of *Drosophila* saline¹⁸ containing 10 μCi ^3H -tyrosine. In c, the incubation medium also contained 10 mM nonradioactive tyramine, which blocks the conversion of the ^3H -tyramine to octopamine. The tritiated metabolites (DA, dopamine; oct, octopamine; tyra, tyramine) were then analysed by high-voltage paper electrophoresis¹⁹. The chemical identity of each peak was confirmed by chromatographing the paper after electrophoresis in a second dimension in two different solvent systems: methyl ethyl ketone/propionic acid/water (25:8:7) or sec-butanol/pyridine/acetic acid/water (30:0.2:2:5). During electrophoresis L-dopa runs so close to tyrosine that any accumulation of L-dopa would not have been detected. The apparent increased height of the dopamine peak in wild-type (a) compared with that of *per*^o (b) is due to the tail of the octopamine region. Electrophoresis of ^3H -octopamine alone yields a profile similar in shape to the octopamine peaks in d with an elevation of the baseline at the dopamine peak by about 15% of the height of the octopamine peak. Flies with a deficiency for *Ddc* (maintained as a heterozygous stock with the balancer chromosome *CyO*:*Df(2L)130*/*CyO*) were mated with flies that had a temperature-sensitive allele of *Ddc* (*Ddc*^{ts1}/*CyO*). We raised the F₁ progeny flies at a permissive temperature (20 °C) until 3 days after eclosion to obtain adults with fully sclerotized cuticles. We then selected the *Ddc*^{ts1}/*Df(2L)130* flies from among the progeny and shifted them to a non-permissive temperature (29 °C) for at least 3 days before biochemical experiments.



synthesized from tyrosine were dopamine, octopamine and tyramine (Fig. 1a). When incubated in tritiated tryptophan, fly brains synthesized and accumulated labelled serotonin (not illustrated).

We examined several behaviourally interesting mutants of *Drosophila*, including those affecting learning, for their ability to synthesize and accumulate labelled monoamine transmitters when incubated with tritiated precursors. One mutant that had a clearly abnormal monoamine profile was *per*^o, previously isolated in a screen to select for mutants lacking normal cir-

cadian rhythms². Two other alleles of the same gene locus also alter these rhythms, producing abnormally long (28 h) or short (19 h) circadian cycles²⁻⁴.

When *per*^o brains were incubated in ^3H -tyrosine, they accumulated less than one-third as much ^3H -octopamine and ^3H -tyramine as wild-type brains, whereas the amount of dopamine accumulated was about the same (Fig. 1a, b). (The absolute height of the dopamine peak is greater in wild type than in *per*^o, but this is because of the overlap between the trailing edge of the octopamine region and the dopamine peak.) There are several possible explanations for this result: the *per*^o mutation could either decrease the number or vigour of octopaminergic neurones or it could affect any step in octopamine synthesis (tyrosine uptake, tyrosine decarboxylation, tyramine hydroxylation, octopamine storage or octopamine release).

To determine if the *per*^o mutation affects any of the enzymes involved in octopamine synthesis we first established (Fig. 1c) that *Drosophila* synthesizes octopamine by the pathway shown in Fig. 2. We then measured separately the activities of the two enzymes required for octopamine synthesis. *per*^o brains had normal levels of tyramine β -hydroxylase activity (not illustrated) but decreased levels of tyrosine decarboxylase activity (Table 1). Reduced levels of tyrosine decarboxylase would account for the observed decrease in accumulation of both octopamine and tyramine. We measured tyrosine decarboxylase activity in three other *per*^o stocks that had different genetic backgrounds and all three strains had less than 50% of the wild-type activity. Other mutant alleles of the *per* locus also showed reduced tyrosine decarboxylase activity: *per*^{long} had 50% of the wild-type activity and *per*^{short} 60%.

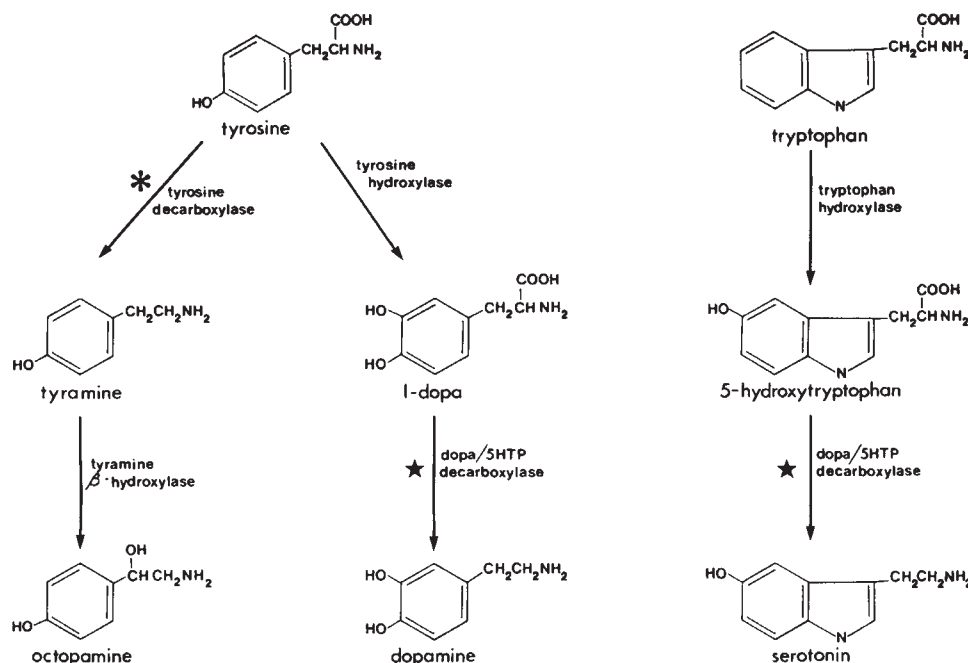
The finding that dopamine synthesis was normal in *per*^o brains suggests that the amino acid decarboxylase used in the synthesis of dopamine must be different from the tyrosine decarboxylase used in the synthesis of octopamine. Wright and his colleagues have determined the genetic locus that codes for dopa decarboxylase and have isolated several mutant alleles and deficien-

Table 1 Tyrosine, L-dopa and 5-hydroxytryptophan decarboxylase activities in different mutants as a percentage of the wild-type activity

	Tyrosine decarboxylase	Dopa decarboxylase	5-HTP decarboxylase
$\frac{+}{+}, \frac{+}{+}$ (wild-type)	100 $\pm$ 4	100 $\pm$ 19	100 $\pm$ 6
$\frac{\text{per}^o}{\text{per}^o}, \frac{+}{+}$	35 $\pm$ 8	83 $\pm$ 19	110 $\pm$ 6
$\frac{+}{+}, \frac{\text{Ddc}^{ts1}}{\text{Df}(2)130}$	72 $\pm$ 7	1.0 $\pm$ 0.1	12 $\pm$ 11
$\frac{\text{per}^o}{\text{per}^o}, \frac{\text{Ddc}^{ts1}}{\text{Df}(2)130}$	33 $\pm$ 4	1.1 $\pm$ 1.4	

Decarboxylase activity was measured by incubating head homogenates with ^3H -tyrosine, ^3H -L-dopa or ^3H -5-hydroxytryptophan and then separating precursor from products by high-voltage electrophoresis. Incubation mixtures (20 μl total) contained 0.2 M sodium phosphate pH 7.2, 0.1 mM pyridoxal phosphate, 2 mM tyramine, 2 mM dopamine, 2 mM serotonin, 5 μCi tritiated precursor and either 10 μl (for tyrosine and 5-hydroxytryptophan decarboxylase determinations) or 5 μl (for L-dopa decarboxylase determinations) tissue homogenate (1 head per 10 μl). Incubations were for 30 min at 25 °C. In these conditions the decarboxylation reactions were linear with time and amount of homogenate.

Fig. 2 Pathways for synthesis of octopamine, dopamine and serotonin^{7-9,20}. The asterisk indicates a reaction partially blocked by the *per*^o mutation, and the stars indicate reactions blocked by *Ddc* mutations.



cies for this locus (*Ddc*)^{5,6}. Dopa decarboxylase is present in both cuticle and brain and this activity is reduced by mutations in the *Ddc* locus^{11,12}, but the effect of *Ddc* mutations on neurotransmitter synthesis has not been explored. Dopamine is an essential intermediate in cuticle sclerotization, and so, in order to be able to study the neurochemistry and behaviour of strains with severe reductions in dopa decarboxylase levels, we used temperature-sensitive alleles of *Ddc* in combination with a deficiency for the region containing the *Ddc* locus (*Df(2L)130*). These flies were raised at a permissive temperature (20 °C) until their cuticles were fully sclerotized and then shifted to a non-permissive temperature (29 °C) 3 days before biochemical testing. When the brains of *Ddc*^{ts1}/*Df(2L)130* flies were incubated in ³H-tyrosine they showed no detectable dopamine synthesis but normal amounts of octopamine and tyramine (Fig. 1d). When incubated with radioactive tryptophan, they showed no synthesis of serotonin whereas both wild-type and *per*^o brains showed serotonin synthesis.

The results with *Ddc* mutants again suggest that wild-type flies have two different amino acid decarboxylases, one that decarboxylates tyrosine and another that decarboxylates both L-dopa and 5-hydroxytryptophan. We tested this directly by measuring each decarboxylase activity in whole head homogenates in the different mutants. Table 1 shows that *per*^o head homogenates had reduced tyrosine decarboxylase activity but normal L-dopa and 5-hydroxytryptophan decarboxylase activities. In contrast, *Ddc*^{ts1}/*Df(2L)130* homogenates showed almost undetectable levels of L-dopa- and 5-hydroxytryptophan-decarboxylase activities but almost normal levels of tyrosine decarboxylase activity. The finding that both L-dopa and 5-hydroxytryptophan decarboxylase activities were abolished by mutations in the structural gene for dopa decarboxylase implies that both substrates are decarboxylated by the same gene product.

Purified *Drosophila* dopa decarboxylase will decarboxylate tyrosine, but it has a much lower affinity for tyrosine than for L-dopa^{13,14}. Therefore it is possible that the small amount of tyrosine decarboxylase activity remaining in *per*^o flies is due to the activity of the dopa decarboxylase enzyme on an anomalous substrate, tyrosine. To assess this possibility we tested flies mutant for both *per*^o and *Ddc*, that is, *per*^o/*per*^o; *Ddc*^{ts1}/*Df(2L)130* (temperature shifted as described in Fig. 1). These flies showed the same 35% residual tyrosine decarboxylase activities as did *per*^o/*per*^o flies not mutant at the *Ddc* locus (Table 1). Therefore, the residual tyrosine decarboxylase

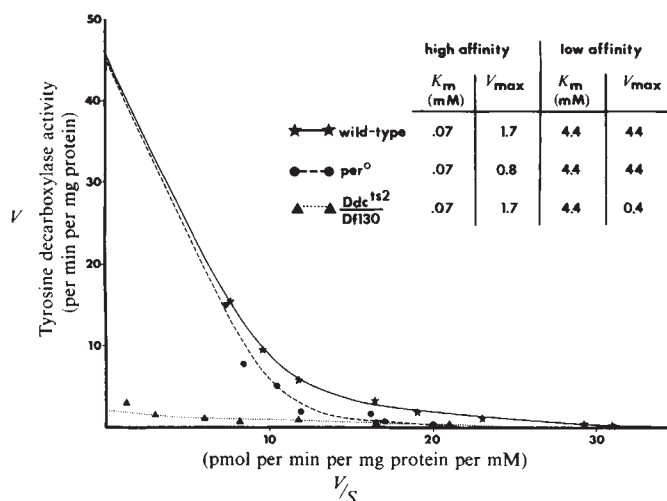


Fig. 3 Eadie-Hofstee plot^{21,22} of tyrosine decarboxylase kinetics: wild-type tyrosine decarboxylase activity has both high affinity ($K_m = 0.07$ mM) and low affinity ($K_m = 4.4$ mM) components. Tyrosine decarboxylase activity was measured by the organic extraction method of McCaman *et al.*²³. Incubation conditions were identical to those described in Table 1 legend except that instead of tritiated precursors, incubations contained ¹⁴C-tyrosine (specific activity = 100 Ci mol⁻¹) at concentrations from 0.01 to 3 mM. Protein was determined by the method of Lowry²⁴. The kinetic parameters were determined by assuming that the K_m s for the two components were the same for all three mutants and then calculating the V_{max} values by using a linear least-squares curve fitting program²⁵. The lines were calculated from the parameters listed.

activity in *per*^o flies is not due to dopa decarboxylase activity, and the *per*^o mutation must not completely eliminate tyrosine decarboxylase activity.

The reason we did not see any tyrosine decarboxylation by the enzyme dopa decarboxylase in the experiment shown in Table 1 is probably that the assays were done at 8×10^{-6} M tyrosine, a concentration well below the K_m of dopa decarboxylase for tyrosine. At higher concentrations of tyrosine, however, the action of both decarboxylases was evident, in that tyrosine decarboxylation showed biphasic kinetics (Fig. 3). Furthermore, the two mutations reduced different components of this activity. In extracts from *per*^o flies the high affinity component was reduced, whereas in extracts from *Ddc*^{ts1}/*Df(2L)130* flies the low affinity component was missing.

If *per*^o is a weak allele of the structural gene for tyrosine decarboxylase, one would expect tyrosine decarboxylase activity to be reduced more severely by a deficiency of the *per* locus, and to be elevated by a duplication of the gene. Neither effect was observed. We measured tyrosine decarboxylase activities in flies heterozygous for *per*^o and three different deficiencies that delete the *per* locus: *Df(1)w¹¹/per^o*, *Df(1)w¹²/per^o* and *Df(1)64c4/per^o* (ref. 15). All of the deficiencies had the same level of tyrosine decarboxylase activity as did *per^o/per^o* flies, but not lower levels. These results suggest that *per^o* is a null mutation, that is, it has the same effect as a deficiency of the gene. (*per^o* likewise acts as a null mutation in its behavioural phenotype⁴.) We found that a duplication of the *per* region (attached to the Y chromosome (*w⁺Y*)) in combination with a *per^o* mutation on the X chromosome elevated tyrosine decarboxylase activity to wild-type levels, but the duplication in combination with the wild-type allele did not further elevate the enzyme levels above the wild-type levels. Thus, tyrosine decarboxylase activity was not directly correlated with the number of *per⁺* gene copies and it therefore seems unlikely that the *per* locus codes for the enzyme tyrosine decarboxylase. Apparently, *per* mutations reduce enzyme levels indirectly, perhaps by affecting octopaminergic neurones.

The use of *Drosophila* mutants has enabled us to distinguish two separate aromatic amino acid decarboxylases. One, which has a high affinity for tyrosine, is used in the synthesis of octopamine and is reduced indirectly by mutations at the *per* locus. The other, encoded by the *Ddc* gene, can decarboxylate all three transmitter precursors but is necessary for the synthesis of only two neurotransmitters: dopamine and serotonin. Octopamine is a major neurotransmitter in many invertebrates¹, but most vertebrates probably do not have specific octopaminergic neurones¹⁶. We expect that the tyrosine-specific decarboxylase described here will be present in the nervous systems of only those organisms that use octopamine as a neurotransmitter.

Many pharmacological experiments indicate that monoamines act as modulators and may modify or determine behavioural states¹⁷. In *Drosophila* we can alter monoamines genetically rather than pharmacologically to explore their role in behaviour. The *per^o* mutation seems to affect octopaminergic function, and *per^o* flies have abnormal biological rhythms but normal learning and memory (not shown). In contrast, the *Ddc* mutation abolishes dopamine and serotonin synthesis but does not affect octopamine synthesis. *Ddc* mutant flies have normal circadian rhythms (unpublished observations) but fail to learn in either negatively or positively reinforced olfactory discrimination tests (B.L.T., M.S.L. and W. G. Quinn, unpublished observations).

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Assignment of the gene for human melanoma-associated antigen p97 to chromosome 3

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p97 is a 97,000 molecular weight cell-surface glycoprotein, which is present in human melanomas but in only trace amounts in normal adult tissues^{1–4}. Amino acid sequence and iron binding studies have shown that p97 is structurally and functionally related to transferrin⁵. Reports that the genes for the transferrin receptor (TR)^{6–8} and possibly transferrin^{9–13} are located on chromosome 3 prompted us to investigate the chromosomal localization of the p97 gene. Our strategy was to characterize interspecies somatic cell hybrids derived from human fibroblasts or lymphocytes for expression of p97 and presence of human chromosomes. Although fibroblasts and lymphocytes express only small amounts of p97^{3,4}, we were able to type the hybrids for p97 by using monoclonal antibodies in highly sensitive and specific immunoassays. Of 14 hybrids, 6 contained chromosome 3 and expressed p97, and 8 were negative for both. We conclude that the p97 gene, like the transferrin and TR genes, is located on chromosome 3.

The first hybrids tested (designated PEP 2B, 6A, 12B, 13B) were obtained by fusing human fibroblasts with HGPRT mouse melanoma cells¹⁴. The hybrids, which had been stored in liquid nitrogen, were expanded in culture and tested for p97 by a binding assay with ¹²⁵I-labelled monoclonal antibody, unlabelled antibody being used as competitor to distinguish specific binding to p97 from nonspecific binding to the cells³. The hybrids were also tested for p97 by a double determinant immunoassay (DDIA), which involves monoclonal antibodies to two distinct epitopes of p97³. The two assays gave similar results but, since the DDIA was more sensitive, it was used for most of the study. Representative data are shown in Table 1. Two hybrids were found to be positive for p97 (300 molecules per cell), and two were negative (fewer than 10 molecules per cell). The human chromosome contents of the four cell lines¹⁴ are shown in Table 2. Only chromosome 3 was present in both p97-positive hybrids and absent from both p97-negative hybrids. The simplest interpretation of the data is thus that the p97 gene resides on that chromosome. The amount of p97 on the positive hybrids, although some three orders of magnitude lower than on melanoma cells, is comparable with levels in fibroblasts³. The hybrids were also tested for TR by a binding assay with ¹²⁵I-labelled monoclonal antibody B3/25 (refs 15, 16). The two hybrids that contained chromosome 3 and

Table 1 Assay of p97 in lysates of mouse × human hybrids by DDIA

Expt	Test sample	Fab 96.5 bound (c.p.m. ± s. e.)	p97 (pg)	Molecules p97 per cell
1	Hybrid PEP 2B	547 ± 9	46	280
	Hybrid PEP 6A	13 ± 0	0	0
	Hybrid 28-4-1	23 ± 0	0	0
	Hybrid 45-1-1	213 ± 4	17	110
	Standard	1,171 ± 60	100	
	Blank	18 ± 3	0	
2	Hybrid 28-1	2,058 ± 15	195	600
	Hybrid 28-4-2	16 ± 1	0	0
	Hybrid 45-1-3	653 ± 12	61	190
	Standard	2,106 ± 108	200	
	Blank	18 ± 4	0	

Cells (2×10^7) were suspended in 1 ml of 50 mM Tris-HCl buffer, pH 8.0, containing 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40 (TNEN) supplemented with 1 mM phenylmethylsulphonyl fluoride. The lysate was centrifuged for 10 min at 3,000g to remove nuclei and then for 1 h at 50,000g. Samples of 50 μ l (expt 1) or 100 μ l (expt 2) were tested for p97. For each 50 μ l of test sample 100 ng unlabelled IgG2a antibody 133.2 (specific for epitope p97^c) and 5 ng Fab fragment of antibody 96.5 (specific for epitope p97^a) labelled with ¹²⁵I to a specific activity of 10^5 c.p.m. per ng were added in 10 μ l TNEN. After 2.5 h at 0 °C formalin-fixed *Staphylococcus aureus* (0.25 mg) were added in 10 μ l TNEN containing 0.7% SDS and the incubation was continued for 10 min. The bacteria were washed four times with 500 μ l TNEN containing 0.1% SDS, and twice with 0.1 M sodium citrate buffer, pH 6.5, containing 0.5% Nonidet P-40. Bound antibody 133.2-p97-Fab 96.5 complexes were eluted in 200 μ l of 0.1 M sodium citrate buffer pH 5.0, containing 0.5% Nonidet P-40 and counted for ¹²⁵I. A melanoma lysate containing 2 ng p97 per ml was used to standardize the assay.

expressed p97 also expressed TR (1,000 to 2,000 molecules per cell), while the other two hybrids expressed fewer than 10 molecules per cell (Table 2).

To verify that the p97 gene is localized on chromosome 3, a second series of interspecies hybrids derived from human peripheral blood lymphocytes was examined. Lymphocytes were cultured with concanavalin A (hybrids 28-1, 28-4-1 and 28-4-2) or pokeweed mitogen (hybrids 45-1-1 and 45-1-3) and then fused with BW5147 mouse thymoma cells¹⁷. The hybrids were selected in medium containing hypoxanthine, aminopterin and thymidine (HAT), cloned on a fibroblast feeder layer, and expanded in medium lacking HAT. Part of the culture was used to prepare a detergent lysate for immunoassay, and another part was taken for karyotype analysis. Chromosome preparations and detergent lysates were made on the same day. Approximately 20 cells from each hybrid were examined for human chromosomes after G-11 and G banding^{18,19}. As well as mouse chromosomes^{7,17}, the hybrids contained several human chromosomes (Table 2).

The hybrids were assayed for p97 by the DDIA (Table 1). Two hybrids were negative for p97 (fewer than 10 molecules per cell) and three were clearly positive (100 to 600 molecules per cell). p97 levels in the positive hybrids were comparable

with levels in the parental lymphocytes (500 molecules per cell). Only chromosome 3 was present in all the p97-positive hybrids and absent from both p97-negative hybrids (Table 2). Hybrid 45-1-3 was particularly informative, since it expressed p97 and contained in addition to chromosome 3 only two other human chromosomes (6 and X), which were present in almost all the other hybrids tested, including those negative for p97. A competition radioimmunoassay for TR²⁰ showed that the three hybrids that expressed p97 and contained chromosome 3 expressed TR (30,000 to 80,000 molecules per cell), whereas the other two hybrids were negative for TR (Table 2).

In addition to the hybrids shown in Table 2, five mouse × human hybrids, of which four were derived from human fibroblasts and one from human lymphocytes, were typed for chromosome 3 by testing them for TR. Four were negative for both p97 and TR, and one was positive for both. Thus of a total of 14 hybrids tested, 6 were positive for both p97 and chromosome 3, and 8 were negative for both ($P = 0.0003$ by Fisher's exact test). This constitutes strong evidence that the p97 gene is located on chromosome 3.

In three independent investigations⁶⁻⁸ the human TR gene has been assigned to chromosome 3. In addition there is evidence that the human transferrin gene is located on that chromosome⁹⁻¹³. It is thus possible that genes coding for several proteins involved in iron transport are located within a small region of chromosome 3. More precise genetic mapping will be required to determine whether this is so.

Although p97 is distinct from both transferrin and TR in antigenicity and tissue distribution, one might still argue that p97 is encoded by either the transferrin gene or the TR gene, being synthesized by alternative (tissue-specific) pathways of transcription, RNA splicing or post-translational modification. Structural data, however, argue against this possibility, since the N-terminal amino acid sequences of p97 and transferrin, though homologous, differ in 5 of 12 positions⁵, and peptide maps of p97 and TR obtained as described²¹ are quite different (data not shown).

Chromosome 3 thus carries the genes for two of the best characterized human tumour-associated antigens, TR and p97, and probably for transferrin, an essential growth factor. Furthermore p97 and transferrin are structurally related to a transforming factor of leukaemic cells²². Rearrangements in chromosome 3 are frequently observed in carcinomas, malignant lymphomas, and monoclonal gammopathies²³, and it is interesting to speculate that they involve the putative iron transport region, particularly since expression of both the p97 and TR genes increases upon malignant transformation^{1,3,4,15,16,24-27}. Mapping of the transferrin, TR and p97 loci within chromosome 3 and comparison with the breakage points in neoplasms will allow this possibility to be tested.

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Table 2 Human chromosome and antigen content of mouse × human fibroblast hybrids

Hybrid	p97 TR		Human chromosomes																							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
PEP 2B	+	+	0	0	25	0	8	67	0	0	0	8	33	25	0	0	0	8	8	25	0	0	0	0	42	
PEP 6A	—	—	34	10	0	17	0	52	24	3	0	14	72	0	24	3	7	10	45	55	10	7	7	0	59	
PEP 12B	+	+	0	8	42	17	8	75	67	0	17	33	50	25	42	0	17	42	33	42	25	17	33	17	58	
PEP 13B	—	—	0	0	0	21	29	25	16	4	4	13	67	29	8	13	4	0	33	54	21	21	13	17	60	
28-1	+	+	0	33	57	0	67	95	76	0	0	81	0	0	0	14	0	5	0	52	0	5	5	0	57	
28-4-1	—	—	0	0	0	0	55	90	0	0	0	45	0	0	0	35	0	10	0	0	0	0	75	0	0	
28-4-2	—	—	0	0	0	0	30	40	35	0	0	40	0	0	0	0	0	0	0	0	0	0	35	0	30	
45-1-1	+	+	0	0	20	0	0	40	25	5	0	0	10	0	0	0	0	0	0	0	0	0	15	0	60	
45-1-3	+	+	0	0	20	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	

Hybrids were typed for p97 and TR by binding assays with radiolabelled antibody and by DDIA or competition radioimmunoassay as described in the text. Presence of human chromosomes is indicated as the percentage of cells in each hybrid containing the respective chromosome.

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Mouse skin carcinomas induced *in vivo* by chemical carcinogens have a transforming Harvey-*ras* oncogene

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Several groups have shown that the malignant phenotype can be transferred to NIH/3T3 fibroblasts by incorporation of DNA isolated from tumour cell lines¹⁻⁵. These studies have demonstrated that the transforming activity of DNA isolated from human bladder, lung and colon carcinoma cell lines is related to an alteration of the cellular homologues of the *ras* genes of Harvey or Kirsten murine sarcoma viruses⁶⁻¹¹. It is, however, unclear what relevance these observations have to the multi-stage nature of tumorigenesis *in vivo*, in which several independent events are required in both humans^{12,13} and experimental animals^{14,15}. The activation of a cellular oncogene in a defined experimental system for the progressive induction of solid tumours has not yet been demonstrated. We report here that high molecular weight DNA from transplanted squamous cell carcinomas induced by sequential treatment of mouse skin with initiators and promoters of carcinogenesis causes morphological transformation of NIH/3T3 fibroblasts at high frequency. The transforming properties are due to the transfer of an activated cellular homologue of the Harvey-*ras* (*ras*^H) oncogene.

Epidermal tumours can be induced by sequential treatment of mouse skin with an initiator (dimethylbenzanthracene, DMBA) and a promoter (12-*O*-tetradecanoyl-phorbol-13-acetate, TPA) of carcinogenesis^{14,15}. The tumours induced by this regimen are primarily benign papillomas, some of which seem to regress spontaneously while others progress to form squamous cell carcinomas¹⁶. Carcinomas which develop on the back skins of mice treated in this way are frequently necrotic and contain dermal fibroblasts interspersed with cells of epithelial origin. We reasoned that the probability of finding an activated oncogene would be optimized by using tumours in which the most malignant cells were selected by subcutaneous

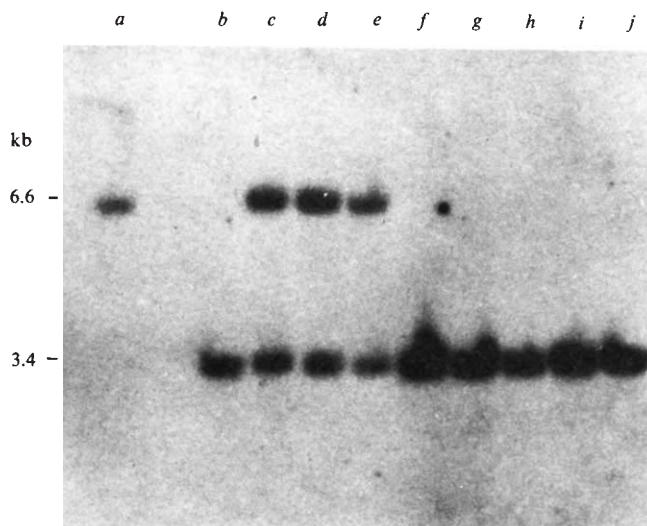


Fig. 1 Southern blot analysis of sequences related to the *ras* gene of Harvey sarcoma virus in NIH/3T3 transformants derived from human or mouse carcinoma DNAs. Solid tumour DNA was isolated by grinding the frozen tissue in liquid nitrogen followed by lysis in 5 M guanidinium thiocyanate, 50 mM Tris-HCl (pH 7.0), 50 mM EDTA and 5% (v/v) 2-mercaptoethanol. The DNA was spun for 24 h through a cushion of 5.7 M CsCl, 50 mM EDTA (pH 7.0), precipitated with ethanol and further purified by treatment with RNases A and T1, protease K and extraction with phenol/chloroform. The tumours were induced on the back skins of NMRI mice by sequential treatment with DMBA and TPA¹⁴⁻¹⁶ and were propagated by subcutaneous injection into a syngeneic host. DNA was isolated from tissue culture cells by direct lysis in buffer containing 5 M guanidinium thiocyanate, followed by further purification as above. High molecular weight DNA (20 µg) was digested with *Bam*HI and electrophoresed through 0.8% agarose gels, blotted on to nitrocellulose and hybridized in stringent conditions (50% formamide, 5×SSC, 43°C) with a nick-translated *ras*^H-specific probe¹⁵ (BS9). Blots were washed to a final stringency of 0.5×SSC at 65°C for at least 1 h and exposed to Kodak film in the presence of intensifying screens for 2 days at -70°C. Fragment sizes were estimated by comparison with co-electrophoresed *Eco*RI-digested λC1857 DNA. DNAs were isolated from the following sources: *a*, EJ bladder carcinoma; *b*, NIH/3T3 cells; *c-e*, primary transformants obtained by transfection with EJ DNA; *f-j*, primary transformants obtained by transfection with mouse carcinoma DNA.

transplantation *in vivo*. Histological examination of these tumours showed that they were highly malignant squamous cell carcinomas which retained the capacity to keratinize (K. Goertler and H. Loehrke, personal communication).

Preliminary transfection studies indicated that DNA from three out of three transplanted carcinomas had the capacity to induce morphologically transformed foci in NIH/3T3 cells at a frequency only slightly lower than DNA from the human EJ bladder carcinoma cell line (Table 1). First-stage foci induced by the EJ DNA were picked and characterized with respect to morphology and the presence of human DNA sequences which hybridized with a cloned *Alu*-family sequence³ (data not shown). In this way, criteria were established which allowed the selection of foci induced in NIH/3T3 fibroblasts by the mouse carcinoma DNA.

It has previously been shown that human cell lines derived from carcinomas contain activated copies of the *ras* genes of Harvey or Kirsten sarcoma viruses¹⁻¹¹. The DNAs from mouse carcinoma-induced foci were therefore initially probed with a nick-translated clone containing part of the *ras*^H oncogene¹⁷ (Fig. 1). Digestion of human DNA with *Bam*HI generates a restriction fragment of 6.6 kilobases (kb) which contains the human cellular homologue of the *ras*^H gene^{6,7}. In a similar digest of mouse DNA, the *ras*^H gene migrates as a 3.4-kb fragment. Both fragments were detected in foci induced by DNA from the human EJ cell line (Fig. 1, lanes *c-e*) whereas those induced by the mouse tumour DNA contained only the

Fig. 2 Detection of sequences related to the ras^H gene in primary and secondary NIH/3T3 transformants derived from mouse carcinoma DNA. The DNA samples (20 μ g) were digested with *Eco*RI, run on a 0.6% agarose gel, blotted and hybridized with a ras^H -specific probe as described in the legend to Fig. 1. DNAs were isolated from: a, human EJ bladder carcinoma cells; b, NIH/3T3 cells; c, d, primary transformants obtained by transfection with EJ DNA; e-i, primary transformants obtained by transfection with mouse carcinoma DNA; j-n, secondary foci obtained after transfection with DNA from 2 different primary foci. The DNA samples in lanes a-i or j-n were run on separate gels.

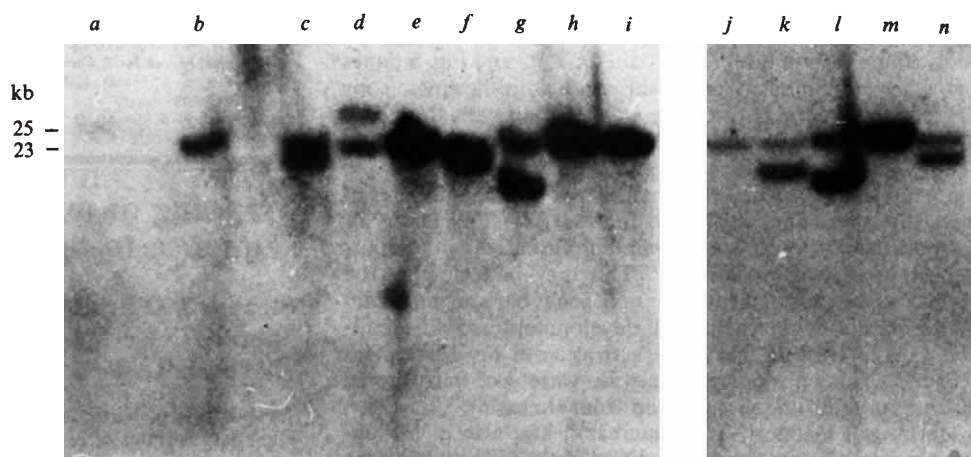


Table 1 Transforming activity of DNA from mouse skin carcinomas

Donor DNA	Total foci/ total recipient cultures	Foci per μ g DNA
Human EJ bladder carcinoma	18/5	0.09
Mouse liver	0/8	—
Mouse brain	0/3	—
Mouse embryo	0/5	—
Mouse carcinoma I	8/4	0.05
Mouse carcinoma II	8/5	0.04
Mouse carcinoma III	12/4	0.08

NIH/3T3 cells were seeded at a concentration of 2×10^6 cells per 75-cm² dish and transfected 1 day later with 40 μ g high molecular weight DNA as a calcium phosphate precipitate^{1,2}. The DNA remained in the cultures for 16 h, after which the cells were maintained in Special Liquid Medium (Flow) supplemented with 5% calf serum and changed at 2–3-day intervals. Foci were scored after 15–18 days in culture. The total number of foci per μ g input DNA obtained with EJ bladder carcinoma DNA is two- to threefold lower than that reported by others^{3,4,23}. Subsequent results have shown that efficiency of transformation is highly dependent on the combination of the NIH/3T3 fibroblast strain used and the type of serum (J. Neil, personal communication).

3.4-kb fragment (lanes f–j). Because foci induced by the mouse carcinoma DNA had a particularly intense 3.4-kb band, suggesting the presence of an additional copy of the ras^H gene, further digestions were carried out with *Eco*RI, which generates a 23-kb ras^H -containing fragment from normal mouse DNA. We reasoned that serial transfection of DNA from the primary foci might ultimately allow the detection of an additional ras^H gene as a restriction fragment smaller than the normal 23-kb size. We found that even some of the primary focus DNAs showed an additional *Eco*RI fragment which hybridized with the ras^H probe (Fig. 2, lanes f, g). Of 11 primary foci probed in this way, 3 showed an additional *Eco*RI fragment containing an extra ras^H gene. The remainder showed evidence of increased ras^H gene copy number by virtue of the high intensity of hybridization with the 'normal' 23-kb fragment. After secondary transfections using DNAs from several primary foci, the frequency of detection of an abnormal restriction fragment containing the ras^H gene increased. Of the five independent secondary focus DNAs shown in Fig. 2 (lanes j–n), three had a smaller *Eco*RI fragment containing the additional ras^H gene. No indication of increased copy number or additional restriction fragment containing the ras^H gene was detected in DNAs from spontaneous foci obtained from the same transfection assays (data not shown).

It remained formally possible that the additional copy of the ras^H gene detected in the induced foci had arisen as a result of duplication of the endogenous ras^H gene of NIH/3T3 cells

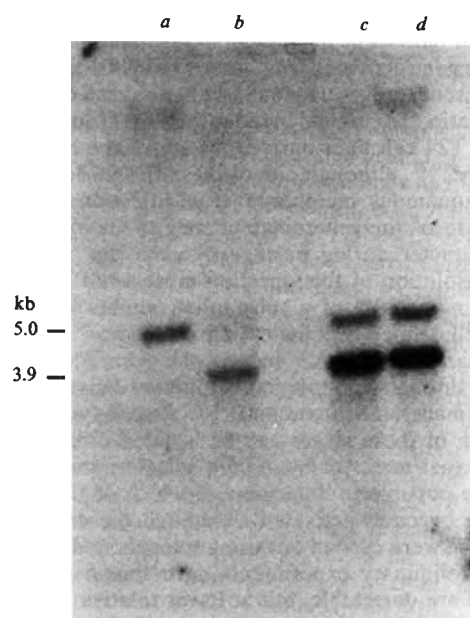


Fig. 3 A polymorphism in the mouse carcinoma ras^H gene is transferred to NIH/3T3 transformants on transfection of mouse carcinoma DNA. Southern blot analysis was carried out on the ras^H genes of NIH/3T3 cells and mouse carcinoma DNA after digestion with the following enzymes: *Bam*HI, *Hind*III, *Eco*RI, *Bgl*II, *Xba*I, *Pst*I, *Pvu*I, *Pvu*II, *Hinc*II, *Kpn*I, *Sst*I, *Sma*I and *Xho*I. Only the *Xho*I digest showed a clear polymorphism which could distinguish between the NIH/3T3 and tumour DNAs. The figure shows a Southern blot of DNAs from the following sources after digestion with *Xho*I and hybridization with the ras^H -specific probe: a, NIH/3T3 cells; b, mouse carcinoma DNA; c, d, primary NIH/3T3 transformants after transfection with mouse carcinoma DNA.

and not by incorporation of exogenous DNA from the tumour. To eliminate this possibility, DNAs from NIH/3T3 cells and from the original tumour used for transfection were digested with a series of restriction endonucleases and hybridized on Southern blots with the ras^H -specific probe to look for a polymorphism characteristic of the tumour DNA. It was found that digestion with *Xho*I generated a restriction fragment of approximately 3.9 kb which was present only in the mouse tumour DNA and not in NIH/3T3 fibroblasts (Fig. 3, lanes a, b). Probing a similar digest of DNA from foci induced by the tumour showed clearly the presence of the NIH/3T3 background ras^H gene as a restriction fragment of about 5 kb and in addition, the 3.9-kb band diagnostic of the exogenous ras^H

gene from the tumour (Fig. 3, lanes c, d). Some evidence for amplification of the tumour *ras*^H gene in the NIH/3T3 transformants can be seen in both Fig. 2 (lanes k, l, n) and Fig. 3 (lanes c, d), where the intensity of hybridization with the single copy NIH/3T3 *ras*^H gene is distinctly weaker than with the newly acquired restriction fragment originating from the tumour.

The demonstration of transforming activity of the *ras*^H gene from a mouse skin carcinoma is consistent with the findings of others that oncogenes related to the *ras* genes of Harvey or Kirsten sarcoma viruses are activated preferentially in tumours arising in epithelial tissues^{1-11,18}. This may indicate that the cellular homologues of the *ras*^H or *ras*^K genes have an important role during normal epithelial cell development and differentiation. It is interesting that while activation of the Kirsten-*ras* oncogene has been reported in a wide variety of transformed cell lines or solid tumours derived from the colon^{5,19}, lung^{5,7}, bladder¹⁸, gall bladder¹⁸ and pancreas¹⁸, and also in a rhabdomyosarcoma¹⁸, transforming activity of the Harvey-*ras* gene has been demonstrated only in a bladder carcinoma cell line (EJ or T24)¹¹, and now in a solid mouse skin carcinoma.

However, no obvious correlation exists at present between the histological pattern of the carcinoma (squamous or transitional) and the gene activated (*ras*^H or *ras*^K). While the tumours used in the present study were clearly squamous cell carcinomas producing keratin, the original bladder tumours from which the human EJ or T24 cell lines derived¹¹ were of the transitional epithelium type^{20,21}, although one of these (EJ) has a reported tendency to squamous metaplasia after injection into nude mice²¹. Because of the inherent tendency of tumour cells to change in character during passage *in vitro* (see ref. 21), a satisfactory resolution of this question must await the testing for transforming activity of a substantial number of primary tumours of well defined histological classification.

Chemical carcinogenesis in mouse skin proceeds in well defined stages through a population of initiated cells and benign papillomas to malignant carcinomas¹⁴⁻¹⁶. As cell types characteristic of each of these stages can be isolated either directly from animal experiments or by *in vitro* selection techniques²², it may now be possible to determine at which of these stages the *ras*^H gene becomes activated. Although the experiments described above were carried out using transplanted squamous carcinomas, preliminary experiments have shown that transforming genes are detectable, but at lower relative concentration, in the DNA of two primary carcinomas (A.B. and J. Smith, unpublished results). Similar results have recently been reported by Pulciani *et al.*¹⁸, who demonstrated that a number of primary human tumours have about fivefold lower transforming activity than established cell lines from human lung and colon carcinomas.

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Mapping of an endogenous retroviral sequence to human chromosome 18

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The application of recombinant DNA technologies has allowed the detection of at least three families of moderately repetitive DNA segments in the human genome that are homologous to retroviruses previously isolated from mice and primates¹⁻³. One of these DNA segments has been shown² by nucleotide sequence comparisons to be distantly related to both Moloney murine leukaemia virus (MoMuLV) and the endogenous baboon retrovirus and to have the sequence organization characteristic of an integrated retrovirus. Isolation of the homologous locus from chimpanzee DNA indicated that the integration event preceded the evolutionary divergence of chimpanzees and man. Here we have used a panel of rodent × human somatic cell hybrids to assign the chromosomal localization of this segment, called *ERV1* (endogenous retrovirus-1), to human chromosome 18 (HSA 18).

The origin and biological significance of the multiple copies of DNA sequences homologous to retroviruses in the normal cellular DNA of vertebrates have been biological puzzles since the original descriptions of endogenous proviruses⁴⁻⁶. Endogenous retroviral families have been detected in numerous mammalian species⁴⁻¹¹ but their biological function, if any, is obscure, as is their adaptive or nonadaptive value in evolution, their role in vertebrate development and the mechanism of retroviral amplification in the host genome. Activation of endogenous retroviruses in certain avian, murine and feline leukaemias has stimulated the formulation of numerous working hypotheses of viral aetiology of cancer^{12,13}. Furthermore, the ability of retrovirus to recombine with and to promote transcription of certain cellular transforming genes (or oncogenes) in several species has led to intensive analyses of these loci in molecular and genetic terms¹⁴⁻¹⁶. Although endogenous retroviruses are non-pathogenic by themselves, they represent a form of mammalian transposable element which on activation becomes capable of both recombination with other cellular genes and reintegration into new chromosomal locations. A rather striking example of apparently pathological recombination is observed in the high leukaemia inbred mouse strain, AKR. AKR mice develop a preleukaemic viraemia including an amphotropic (MCF) virus which is generated *de novo* by a recombination between endogenous ecotropic and xenotropic envelope virogenes^{17,18}. In addition, it seems that retroviruses have been rather mobile during mammalian evolution. It has been shown that several of the presently known endogenous proviruses have been transferred between the germ lines of distantly related biological taxa^{8,9,19}.

The search for retrovirus in human neoplastic disease and in human tissues has only recently been successful^{20,21}. Several isolates of a human retrovirus have been associated with certain T-cell leukaemias, but this virus is not endogenous to normal human DNA²². More recently, three laboratories have independently described apparently different families of human DNA

Table 1 Chromosomal and *ERV1* phenotype of rodent × human somatic cell hybrids

Hybrid	Human chromosome																						X	ERV1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		
80H7A	-	M	-	-	+	-	+	-	-	-	+	+	-	-	+	+	-	+	-	-	+	-	+	+
80H12A	-	-	-	+	-	+	-	P	M	-	-	-	-	M	+	-	-	+	-	-	-	-	+	+
81P1A	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+
81P2A	-	+	+	+	-	+	+	-	+	M	+	+	+	M	+	+	-	+	+	P	+	+	+	+
81P3A	-	-	+	+	-	-	-	-	-	P	+	-	-	+	+	-	-	+	-	+	-	+	+	+
81P6A	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+	-	+	M	M	+	+
81P7A	P	-	-	M	-	-	P	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	+
81P17A	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	P	-	+	-	M	M	+	+	+
81P18A	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	+	M	-	-	-	+	+
70M4A	+	+	+	+	-	+	+	+	+	+	+	+	P	+	-	P	+	+	+	M	P	P	+	+
70M8A	-	-	+	+	+	+	+	-	+	+	+	-	-	+	-	+	-	+	-	-	+	+	+	+
70M10A	-	-	-	M	-	+	+	-	+	+	M	M	M	+	-	-	M	+	+	-	M	M	+	+
70M11A	-	-	+	-	-	M	+	-	M	-	-	-	-	+	M	-	-	+	-	+	+	-	+	+
70M16A	+	+	+	+	+	+	+	+	+	+	P	P	P	+	M	M	M	+	M	M	M	M	+	+
80H2A	-	-	-	-	-	+	-	+	+	-	-	P	+	+	+	-	-	-	M	+	-	+	+	-
80H5A	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	M	-	-	+	-
80H6A	-	-	-	P	-	+	-	-	-	-	-	-	-	P	-	+	-	-	-	P	P	M	P	-
80H8C	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	+	-
80H10A	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
80H11A	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
81P4A	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-
81P5A	-	-	-	M	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-
81P11A	-	-	-	+	-	-	-	-	M	-	-	-	P	+	+	+	-	-	+	-	+	+	+	-
81P14A	-	-	-	-	-	-	-	-	+	-	+	-	P	-	+	-	-	-	+	-	+	+	-	-
70M1A	-	-	-	+	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	+	-	+	-
70M2A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
70M3A	-	-	-	M	-	M	-	-	-	-	-	-	P	-	-	M	-	-	-	-	+	-	+	-
70M5A	-	-	-	-	-	M	+	-	-	-	-	-	-	-	-	-	-	-	P	-	+	P	+	-
70M6A	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
70M7A	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
70M9A	-	-	-	+	-	+	+	+	+	M	+	-	-	+	-	-	+	-	+	M	+	M	+	-
70M12A	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	+	-
70M13A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
70M15A	-	-	-	-	-	M	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	P	+	-
% Discordancy with <i>ERV1</i>	35	30	29	29	35	24	24	32	31	28	33	34	41	32	38	21	38	0.0	32	27	45	31	58	

Chromosomal and *ERV1* phenotype of rodent × human somatic cell hybrids; the 80 and 81 series are hybrids of E36 (Chinese hamster) × fresh human lymphocytes; the 70 series are RAG (mouse) × fresh human lymphocytes. Chromosome scores indicate the consensus result of G-banding and isozyme scores. P indicates present but at a low frequency; M represents an uncertain negative (for example, 1 chromosome seen in 20 spreads or the presence of a broken version of the chromosome). Thirty-six isozyme systems diagnostic for each human chromosome were run on each hybrid. The details of isozyme analysis of human hybrids have been described elsewhere⁴⁹. The isozymes and their chromosome assignments as follows. HSA 1, phosphoglucomutase-1, 6-phosphogluconate and peptidase-C; HSA 2, isocitrate dehydrogenase (soluble), malate dehydrogenase (soluble) and acid phosphatase-1; HSA 3, amino acylase-1; HSA 4, peptidase-S and phosphoglucomutase-2; HSA 5, hexosaminidase-B; HSA 6, glyoxylase, phosphoglucomutase-3 and malic enzyme-1; HSA 7, β -glucuronidase; HSA 8, glutathione reductase; HSA 9, adenylate kinase; HSA 10, inorganic pyrophosphatase and glutamate oxaloacetate transaminase; HSA 11, lactate dehydrogenase-A and acid phosphatase-2; HSA 12, triose phosphate isomerase, lactate dehydrogenase-B and peptidase-B; HSA 13, esterase-D; HSA 14, purine nucleoside phosphorylase; HSA 15, mannose phosphate isomerase and hexosaminidase-A; HSA 16, diaphorase-4; HSA 17, galactokinase; HSA 18, peptidase-A; HSA 19, glucose phosphate isomerase (GPI) and peptidase-D; HSA 20, adenosine deaminase; HSA 21, superoxide dismutase-1; HSA 22, aconitase-2; HSA X, glucose-6-phosphate dehydrogenase.

sequences which are distantly related to replication-competent retroviruses isolated from other mammals¹⁻³. The genetic localization of these endogenous virogenes would assist in the detection of any *cis* regulation of linked cellular genes as well as provide a perspective for genetic analysis of transformation. Further, the comparative chromosomal position of homologous proviral genomes in related vertebrate species should be useful in reconstructing the evolutionary origins of the particular retroviral genome^{2,23}.

Bonner *et al.*² have described a 20-kilobase (kb) segment of the human genome containing an 8-kb region with appropriately spaced DNA sequence homology (51–57%) to the *gag* and *pol* genes of MoMuLV. A 480-base pair (bp) sequence which structurally resembles a retroviral long terminal repeat (LTR) was detected 3' to these genes in a position predicted if the sequence were a functional retrovirus. However, the LTR sequence that would be the 5' end of the integrated genome is absent; thus there is no viral promoter sequence. This human retroviral sequence had approximately the same degree of sequence homology (62%) with certain regions of the *pol* gene of baboon endogenous virus (BaEV) as it did with MoMuLV (57%). Thus, the human sequence is almost equally related to

BaEV and MoMuLV viruses but less related to those two viruses than they are to each other. We describe here the chromosome mapping of this human proviral DNA segment.

Somatic cell hybrids were constructed between fresh human lymphocytes from a healthy female (LLL) and the rodent cells, mouse RAG (ref. 24) and Chinese hamster, E36 (ref. 25). The rodent cells were mutant in the *HPRT* gene, which allowed selection on hypoxanthine-aminopterin-thymidine (HAT) medium²⁶. Nine independent fusions were performed using PEG 1000, and 249 hybrids were derived (161 E36 × LLL and 78 RAG × LLL). Extracts of each of these hybrids were typed for diagnostic isozyme markers (~36; see Table 1) previously assigned to individual human chromosomes²⁷⁻²⁹. These hybrids retained the entire rodent genome but segregated human chromosomes in different combinations. Two mapping panels (30 hybrids with E36 and 16 hybrids with RAG) were chosen with special attention given to selecting hybrids having low numbers of human chromosomes, but with strong isozyme signals for the human parent. The hybrids were expanded on HAT medium, and high-molecular weight DNA was extracted at the same cell passage from which an isozyme extract and karyotypic spread were prepared. G-11 staining was performed

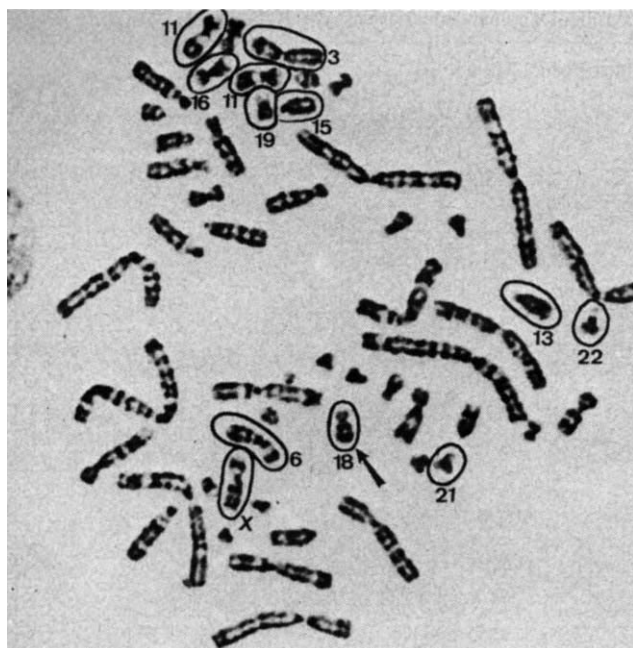


Fig. 1 Trypsin G-banded karyotype of hybrid 81P2A; human chromosomes are circled and HSA 18 is indicated by an arrow. Metaphase chromosomes were banded by using trypsin followed by Giemsa staining as described elsewhere^{23,31}.

on each hybrid in the panel to discover hybrids having numerous chromosome rearrangements; this procedure stains human chromosomes light blue and rodent chromosomes magenta³⁰. Hybrids having appreciable human chromosome rearrangements or interspecific chromosome translocations were discarded. Each hybrid was G-banded^{23,31} to determine the human chromosome complement (Fig. 1).

An 8.0-kb *EcoRI* fragment characteristic of the *ERV1* locus can be detected in human DNA by hybridization to a nick-translated probe derived from a 2.3-kb *EcoRI* fragment of the human clone HC-20 containing portions of the polymerase and putative envelope genes. This is the only fragment detected using high stringency hybridization conditions with DNA from five different humans. Furthermore, it is present in the homologous chimpanzee clone, CH2, and in a previously undescribed human clone, HC-62, which extends the 3' end of the sequence representing the *ERV1* locus by 5.8 kb. Low stringency hybridization allows the detection of at least 15 *ERV1*-related *EcoRI* fragments in human DNA and in human × rodent hybrid DNAs while none are detected in E36 DNA (Fig. 2) or RAG DNA (not shown). The isozyme, karyotype and *ERV1* signal for each of 32 hybrids were determined. The frequency of concordance between *ERV1* for each human isozyme and each chromosome was calculated with computer assistance as described elsewhere²³. The composite chromosome and isozyme phenotype of each of the 32 hybrids is presented in Table 1, together with the frequency of concordance of each chromosome with detection of *ERV1*. *ERV1* showed perfect concordance (100%) with human chromosome 18 (HSA 18) and *PEPA* (peptidase-A; Fig. 3), an isozyme marker previously mapped to this chromosome. Each of the remaining 22 human chromosomes showed high discordance with *ERV1* (21–58%). These results thus assign the endogenous retroviral sequence, *ERV1*, to human chromosome 18.

Endogenous retroviral genes have been mapped in the mouse using sexual as well as somatic cell genetic crosses. Almost 20 endogenous retroviral loci have been shown to distribute throughout the mouse genome in various inbred strains^{32–34}. In man, however, *ERV1* represents the first endogenous provirus

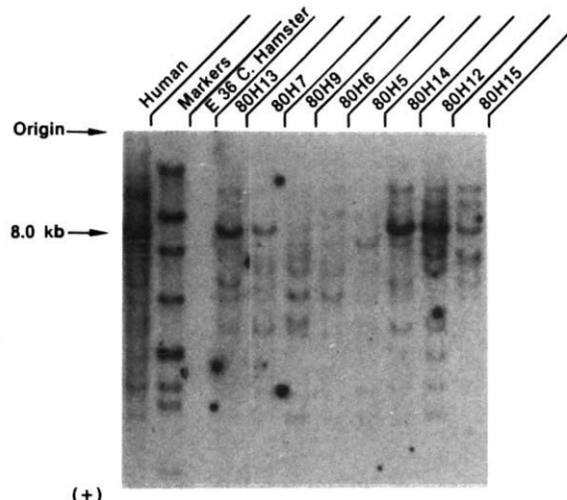


Fig. 2 Hybridization of labelled cloned human DNA to *EcoRI* restriction digests of human, hamster and human × hamster DNAs. Restriction digests containing 2–5 µg DNA were electrophoresed in 0.7% agarose gels, transferred to nitrocellulose filters and hybridized at low stringency as described previously². The probe was a nick-translated plasmid containing the 2.3-kb *EcoRI* fragment of HC-20 (ref. 2) labelled to a specific activity of $\sim 10^8$ c.p.m. µg⁻¹. The 80H series of hybrids are all human × E36 hybrids. All lanes contained comparable amounts of DNA as indicated by ethidium bromide fluorescence and by comparable intensities of at least some bands in all hybrid DNAs. Most of the hybrid DNAs were scored for *ERV1* using high stringency hybridization conditions². The markers are end-labelled *HindIII* fragments of λDNA and *TaqI* fragments of φX174 DNA.

to be chromosomally localized. Recently, several additional genes which relate to neoplastic transformation have been mapped in man^{35–38}. Cellular transforming genes or oncogenes are a class of evolutionarily conserved vertebrate DNA loci which have been identified as recombinational inserts of acute transforming retroviruses in several species^{14–16}. The acquisition of these oncogenes by competent retroviruses renders them capable of oncogenic transformation of tissue culture cells and able to induce various neoplasms *in vivo* with a short latency period. Several human homologues of oncogenes have been mapped to various human chromosomes including *myb* (HSA 6), *myc* and *mos* (HSA 8), *abl* (HSA 9), *fes* (HSA 15), *src* (HSA 20) and *sis* (HSA 22)^{35–38}. Furthermore, a specific requirement for the gene *BEV1* on HSA 6 has been demonstrated for the exogenous infection and replication of BaEV in human cells^{39–41}.

Many human chromosomes are also known to be nonrandomly associated with certain types of human cancers^{42–45}. Klein⁴⁴ has suggested that certain human cancers may result from translocation of transforming genes to chromosomal positions where the regulation of transcription is altered. A particularly cogent demonstration of this hypothesis has developed from the genetic analysis of *c-myc*, the human cellular homologue for the transforming gene of avian myelocytomatosis virus, MC29 (refs 46–48). The *c-myc* gene is normally localized on the long arm of chromosome 8 (q24–qter); this precise region is broken and translocated to chromosome 14 in 90% of all Burkitt lymphomas. The remaining 10% of Burkitt tumours have the *c-myc* region transposed to either chromosome 2 or 22. These three chromosomes are the site of the immunoglobulin genes and the placement of *c-myc* transcription under the *cis* control of immunoglobulin heavy-chain genes (chromosome 14) in certain Burkitt tumours has been demonstrated recently^{46–48}.

In a recent report, Yunis found that 100% of the cases with a non-Hodgkin's nodular small cleaved cell lymphoma were characterized by a reciprocal translocation between chromosome 18 (q21.3) and chromosome 14 (ref. 45). The break in chromosome 14 is also in the region of the immunoglobulin

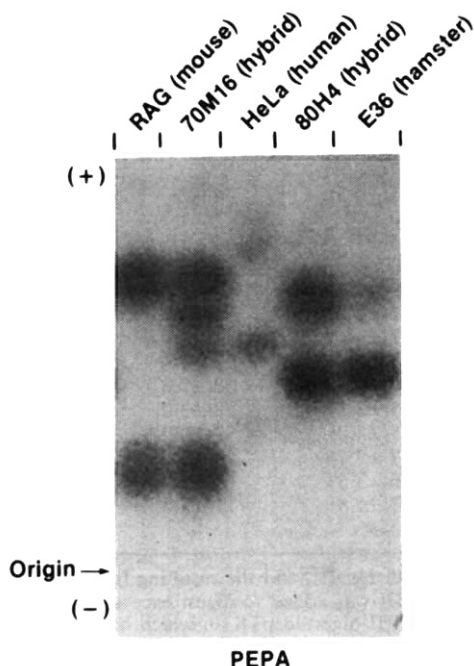


Fig. 3 Electropherogram of peptidase A (PEPA) extracts of mouse (RAG), Chinese hamster (E36), human and hybrid cells known to be positive for HSA 18. Note that mouse-human hybrids are scorable while hamster-human hybrids are not.

heavy-chain gene (q32.3 on the long arm). The possible association of *ERV1*, the heavy-chain genes on chromosome 14 and the neoplasia are areas of considerable interest. The *ERV1* locus contains a sequence homologous to retroviral (LTRs) which contain powerful eukaryotic promoters of transcription. Whether the LTR promoter, the rearrangement of somatic immunoglobulin genes and/or the activation of previously unidentified transforming genes participate in some way in the development of these lymphomas must await both molecular and fine structure mapping of these translocation-bearing tumours.

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Transcriptional control signals in the genome of bovine papillomavirus type 1

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Papillomaviruses cause benign tumours in their natural hosts, which, in some cases, become foci for the appearance and spread of malignant carcinomas¹⁻³. It is therefore of considerable interest to analyse the strategy of gene expression of this group of viruses. Towards this end, we have used a generally applicable technique, in which we monitor the ability of DNA fragments to induce the expression of an inactive thymidine kinase (*tk*) gene, from which the promoter region has been deleted. Using this approach we now report for the first time the location of four transcriptional control sequences in the early region⁴ of the genome of bovine papillomavirus type 1 (BPV-1). Two of the sequences with the characteristics of 'promoter' elements are located proximal to the putative 5' leaders⁵ of the early genes. The third sequence is located within the region coding for the main body⁵⁻⁸ of the early transcripts, and the fourth within the region spanning the 3' end of the early open reading frames and the 5' end of the late ones^{5,8}. The fourth sequence displays the characteristics of an 'enhancer' element; however, unlike previously described 'enhancers'⁹⁻¹³, it is located three to five kilobases (kb) away from the first two promoters and to the 3' side of the third element.

BPV-1 causes benign fibropapillomas (warts) of the skin in domestic cattle¹, and also induces fibrosarcomas in heterologous hosts such as the horse, hamster or pika¹⁴⁻¹⁷. The virus, or its DNA (either from virions or molecularly cloned), are capable of the malignant transformation of *in vitro* cultured bovine epithelial cells^{18,19} and rodent fibroblasts²⁰⁻²⁵. In all cases the viral genome is found as multiple copies, both monomers and oligomers, of circular DNA^{15-17,19,22-27}. Virus antigens and particles are produced in abundance in warts, but only low levels of viral RNA⁵⁻⁷, and no viral antigens or particles^{16,17,19} have ever been detected in tumours induced in heterologous hosts or in transformed cells. Although the lack of an *in vitro* system for productive infection has hindered efforts to analyse gene expression, the viral RNA species present in warts and in

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Table 1 Transformation of LATK⁻ cells by recombinant plasmids containing BPV-1 fragments inserted into the *Hind*III site of pTK10

Donor DNA	Insert†	Orientation‡	No. of TK ⁺ colonies per flask (±s.d.§)			
			0.01	0.1	1	10
1. pTK1		5'-3'	16(±15)	127(±20)	TMTC	TMTC
2. pTK9		3'-5'	—	1.5(±0.5)	34(±2)	—
3. pTK10		—	—	0	1.2(±1.2)	0.8(±1)
4. pTKε1¶	ε-globin promoter	5'-3'	—	25(±1)	164(±1)	—
5. pTKε2¶	ε-globin promoter	3'-5'	—	0	8(±1.0)	—
6. pTKMoLTR1#	MoMuSV LTR	5'-3'	263(±24)	TMTC	TMTC	—
7. pTKMoE1**	MoMuSV enhancer	5'-3'	12(±1)	271(±26)	TMTC	—
8. pTKBV1-189	A	3'-5'	—	17(±4)	81(±10)	TMTC
9. pTKBV1-190	A	5'-3'	—	17(±3)	82(±10)	TMTC
10. pTKBV1-22	C	5'-3'	—	4.2(±1.5)	28(±7.0)	90(±24)
11. pTKBV1-23	C	3'-5'	—	1.3(±1.5)	8(±3.0)	24(±6.5)
12. pTKBV1-94	L	3'-5'	—	6.5(±4.0)	37(±22)	119(±64)
13. pTKBV1-142	L	5'-3'	—	5.7(±2.7)	40(±19)	119(±44)
14. pTKBV1-33	B'	5'-3'	—	10(±3.5)	64(±14)	162(±14)
15. pTKBV1-34	B'	3'-5'	—	3(±1.8)	11(±2.6)	30(±5.5)
16. Salmon		—	—	—	—	0

Ten µg of BPV-1 DNA, excised from pBV1⁴⁴ by digestion with *Hind*III, were digested with *Hae*III, and the resulting fragments ligated to *Hind*III molecular linkers with T4 DNA ligase. After adjusting the buffer, an excess of *Hind*III was added to digest excess linkers, the DNA fragments were separated from the digested linkers on Sepharose CL 6B and ligated to *Hind*III-digested pTK10, which had been previously treated with bacterial alkaline phosphatase. The ligated DNA was used to transfect competent CaCl₂-treated *Escherichia coli* HB101 cells and ampicillin-resistant colonies were screened by *in situ* hybridization⁴⁵ to ³²P-labelled nick-translated⁴⁶ BPV-1 DNA. The positive colonies were grown in L broth containing ampicillin, and plasmid DNA was isolated^{47,48} and analysed. The identity and orientation of the BPV-1 DNA fragments contained in recombinant plasmids were established by restriction enzyme mapping and, after end-labelling with ³²P using polynucleotide kinase, by hybridization to Southern blots⁴⁹ of BPV-1 DNA fragments. Closed circular pTKBV1 plasmids, purified by CsCl buoyant density gradient centrifugation, were used to transfect LATK⁻ cells by the CaPO₄-DNA precipitation technique⁵⁰. LATK⁻ mouse cells were plated at a concentration of 1 × 10⁶ per 25 cm² flask in SF12 medium containing 15% fetal calf serum (FCS). After 24 h, 0.5 ml of precipitated DNA per 5 ml of medium were added, and the cells were incubated for 4 h at 37 °C. The medium was then changed and after 20 h was replaced with HAT selective medium⁵¹ containing 15% FCS. The HAT medium was changed every 3–4 days for up to 3 weeks, when colonies were fixed with 100% ice-cold methanol and stained with 10% Giemsa in distilled H₂O. Only the plasmids positive in the transformation assay are listed. TMTC, Too many to count.

* When 1 µg or less of recombinant DNA were used, 10 µg of salmon sperm DNA were added as carrier.

† The DNA fragments inserted into the *Hind*III site of pTK10. 9–16 contain the *Hind*III–*Hae*III BPV-1 fragments.

‡ Orientation of the inserted fragments relative to the direction of transcription.

§ The numbers are the average of six independent measurements, in three different experiments, except for 2 and 4–7, which are the average of four measurements in two experiments.

|| pTK9 was derived from pTK1 by inversion of the *Bgl*II–*Pvu*II fragment containing the 5' regulatory sequences of the *tk* gene, after insertion of *Hind*III molecular linkers at both restriction sites.

¶ The 197 bp *Bam*HI–*Pvu*II fragment containing the human ε-globin gene promoter³⁵ was inserted using *Hind*III molecular linkers into the *Hind*III site of pTK10.

The 740 bp *Eco*RI–*Sma*I fragment containing the 474 bp 5' terminal portion of the MoMuSV LTR and 5' flanking mink DNA³⁶ was inserted using *Hind*III molecular linkers into an *Eco*RI–*Hind*III deletion of pTK10. Initiation of the *tk* gene transcription is controlled by the MoMuSV 72 bp repeats and the linked consensus promoter sequences³⁷.

** pTKMoE1 was derived from pTKMoLTR1 by removal of the MoMuSV promoter sequences. This leaves the MoMuSV 72 bp repeat linked 5' to the *tk* gene. A more detailed description of both pTKMoLTR1 and pTKMoE1 will be published elsewhere (J.L., D.A.S. and N.M.W., in preparation).

transformed cells have been assigned to specific sub-genomic regions^{5–7}. Both the early (transforming) and late (productive) RNA appear to be transcribed from the same DNA strand. Recently, the nucleotide sequence of BPV-1 DNA has been reported⁸. The sequence analysis confirms the presence of open reading frames on one DNA strand only, thus supporting the unidirectional transcription model.

To identify putative control signals in the viral genome, we investigated the ability of sub-genomic fragments of BPV-1 DNA to replace the promoter region of the herpes simplex virus type 1 (HSV-1) thymidine kinase (*tk*) gene in functional assays. The entire functional promoter, cap site and first 54 bases of the 5' untranslated leader of the *tk* gene are located within the 249 base pair (bp) *Pvu*II–*Bgl*II fragment^{28–31} of pTK1, a previously described plasmid³² containing a 3.58 kb *Bam*HI fragment of HSV-1 DNA³³ inserted into the *Bam*HI site of pAT153³⁴ (Fig. 1). The *Pvu*II–*Bgl*II fragment of pTK1 has been deleted, and replaced with a *Hind*III recognition sequence to produce plasmid pTK10 (Fig. 1). This deletion mutant is inactive in several functional assays for expression of the *tk* gene, but can be reactivated by the insertion into the new *Hind*III site of the 5' control sequences of a number of

eukaryotic genes, providing therefore a generally applicable assay for putative transcriptional control sequences. Thus, the insertion of the 197 bp human ε globin gene promoter³⁵ restores the ability of pTK10 to transform LATK⁻ cells, with an efficiency approximately one-fifth that of the intact *tk* gene in pTK1 (Table 1). The insertion of a 740 bp fragment containing 473 bp of the Moloney mouse sarcoma virus (MoMuSV) long terminal repeat (LTR) element³⁶, including the promoter³⁷ and the 'enhancer' sequences¹², causes the reactivation of the *tk* gene to give approximately a 20-fold increase in transformation efficiency compared with pTK1 (Table 1).

We cloned the unfractionated *Hind*III–*Hae*III double-digest fragments of BPV-1 DNA (Fig. 2) into the *Hind*III site of pTK10 using *Hind*III molecular linkers. With the exception of fragments H, O and P, all the possible fragments were obtained as individual recombinants, which were tested for *tk* gene expression by their ability to convert LATK⁻ cells to the TK⁺ phenotype. Table 1 shows the number of TK⁺ transformants obtained by transfection of LATK⁻ cells with 0.1, 1.0 and 10 µg of pTK1, pTK10 and the pTKBV1 recombinant plasmids. As expected, the number of transformed colonies induced by pTK10 was not significantly greater than the background value

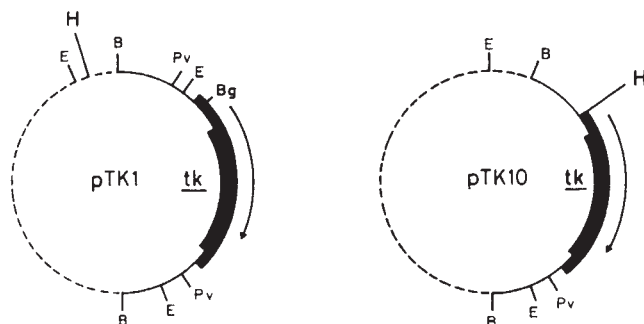


Fig. 1 Physical maps of pTK1 and pTK10. pTK10 was derived from pTK1³² by deletion of the *Hind*III site in the pAT153 moiety, followed by deletion of the 249 bp *Pvu*II-*Bgl*III fragment and replacement with *Hind*III molecular linkers. pTK10 thus contains a new *Hind*III site proximal to the 5' end of the *tk* gene. A more detailed description of pTK10 will be published elsewhere (J.L., D.A.S. & N.M.W., in preparation). The broken line indicates pAT153; the solid line the 3.58 kb *Bam*HI fragment of HSV-1; the outer heavy line the structural *tk* gene; and the inner heavy line the coding sequence. The arrow indicates the direction of transcription. The maps are not drawn to scale and the *Hind*III site is emphasized. E, *Eco*RI; H, *Hind*III; B, *Bam*HI; Pv, *Pvu*II; Bg, *Bgl*III.

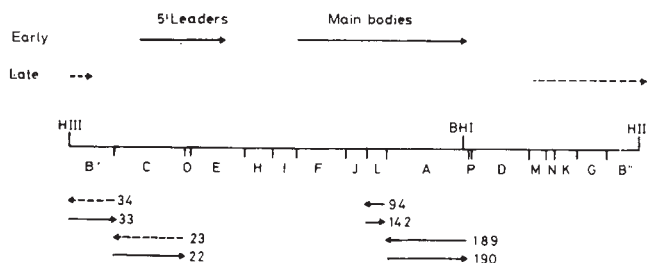


Fig. 2 *Hind*III/*Hae*III restriction map of BPV-1 DNA, and location of the putative transcriptional control regions. *Hae*III cleaves BPV-1 DNA 16 times, and *Hind*III cleaves it once, in the *Hae*III fragment B generating B' and B''. The sizes (bp) of the 17 fragments are: A, 1175; B', 630; B'', 433; C, 978; D, 815; E, 750; F, 675; G, 487; H, 365; I, 340; J, 273; K, 268; L, 259; M, 242; N, 143; O, 70; P, 42. The large fragments were ordered on the genome map by double-digestion with restriction enzymes, and the smaller ones with the aid of the nucleotide sequence of the viral DNA⁸. The *Bam*HI site (BHI) separates the 69% region of the genome containing the early functions⁴, from the region containing the structural genes⁵. The fragments with transcriptional control activity are indicated by the lower arrows. The direction of the arrows indicates the orientation of the fragments cloned in the *Hind*III site of pTK10, relative to the direction of transcription. Solid arrows represent stronger activity than broken arrows. The upper solid and broken arrows are a schematic representation of the early and late transcripts respectively^{5,6}. The late transcripts span the *Hind*III site, but the location of their 5' ends has not been established with certainty.

Table 2 Transformation of LATK⁻ cells by recombinant plasmids containing BPV-1 fragments inserted into the *Hind*III site of pTK1

Donor DNA	Insert	Orientation	No. of TK ⁺ colonies per flask (±s.d.)	
			Amount of DNA used (μg)*	
1. pTK1			0.01	0.1
2. pTK10			11(±5.2)	104(±24)
3. pTK1SV1*	SV40 enhancer	5'-3'	0	0
4. pTK1MoE1†	MoMuSV enhancer	5'-3'	225(±25)	TMTC
5. pTKBV1-201	A	5'-3'	234(±3)	TMTC
6. pTKBV1-202	A	3'-5'	78(±18)	TMTC
7. pTKBV1-203	C	5'-3'	65(±26)	TMTC
8. pTKBV1-204	C	3'-5'	14(±7.0)	86(±11)
9. pTKBV1-205	B'	5'-3'	14(±6.2)	107(±10)
10. pTKBV1-206	B'	3'-5'	9(±4.7)	93(±27)
11. pTKBV1-207	L	5'-3'	13(±4.9)	104(±17)
12. pTKBV1-208	L	3'-5'	12(±6.8)	107(±29)
			13(±6.6)	90(±30)

The pTKBV1 plasmids positive in the transformation assay shown in Table 1 were digested with *Hind*III and the viral DNA fragments were separated from pTK10 through a low-melting point agarose gel. The viral fragments were ligated to *Hind*III-digested pTK1, which had been treated with bacterial alkaline phosphatase. Transfection of competent cells, screening of the colonies, extraction of the plasmid DNA and transfection of LATK⁻ cells were as described in the legend to Table 1. The orientation of the viral fragments in the recombinant plasmids was determined by digestion with restriction enzymes and by heteroduplex mapping.

* The 450 bp fragment containing the transcriptional control elements of SV40 carried in pSO59, was ligated to the *Bam*HI site of pTK1 upstream from the *tk* gene, in a 5'-3' orientation relative to the direction of transcription.

† The *Bam*HI fragment of pTKMoE1 containing the MoMuSV 72 bp repeat, was ligated into the *Bam*HI site of pTK1 upstream from the *tk* gene, in a 5'-3' orientation relative to the direction of transcription. The construction of both pTK1SV1 and pTK1MoE1 will be described in greater detail elsewhere (J.L., D.A.S. and N.M.W., in preparation).

§ As in Table 1.

observed with salmon sperm DNA. In contrast, transfection with pTKBV1-189, -190, -22, -23, -94, -142, -33 and -34 yielded a significant number of TK⁺ transformants. These recombinants contained fragments A, C, L and B' in either orientation. Whereas the number of colonies obtained with fragments A and L was independent of their orientation relative to the *tk* gene, fragments C and B' induced a five times lower number of transformants when oriented 3'-5' to the direction of transcription of the *tk* gene. This is in agreement with the marked reduction in the number of transformants observed with pTK9 and pTKε₂, in which the orientation of the HSV-1 *tk* gene promoter and the human ε-globin gene promoter respectively, is inverted in relation to the direction of transcription

(Table 1). pTKBV1 plasmids containing all the other cloned BPV-1 DNA fragments were totally inactive.

The transformation efficiency of the active pTKBV1 plasmids was considerably lower than the activity of the intact *tk* gene in pTK1. In the best cases the activity was approximately 10-40 times lower than with pTK1, but only 2-4 times lower than with pTKε₁.

Several laboratories have reported recently the presence of so-called enhancer sequences in the genome of papovaviruses⁹⁻¹¹ and retroviruses¹². In artificially constructed systems, these elements increase gene expression when located in either orientation up to several kilobases distal to target genes^{38,39}. For example, when the SV40 or the MoMuSV 'enhancers'^{10,12}

are inserted into the *Bam*HI site upstream from the *tk* gene in pTK1, an approximately 20-fold stimulation of transformation is observed (Table 2). Surprisingly the enhancer elements can also act as 'promoters'. When the MoMuSV enhancer sequence is inserted 5' to the *tk* gene in pTK10, the ability to induce TK⁺ transformants is restored (Table 1). The mechanism for this reactivation is not known, but may be due to the ability of 'enhancer' elements to activate cryptic transcriptional control sequences. To test whether any of the active BPV-1 fragments had 'enhancing' properties, they were excised from the pTKBV1 plasmids with *Hind*III and inserted in both orientations into the *Hind*III site of pTK1. The results obtained when these plasmids were used to transform LATK⁻ cells are shown in Table 2. Only the A fragment significantly increased the transformation frequency and did so in an orientation-independent fashion. Moreover, it also increased the transformation frequency of both RAT4 TK⁻ cells and human 143 TK⁻ cells by the same degree as it did in LATK⁻ cells (unpublished observations). Therefore, fragment A has properties similar to those previously ascribed to 'enhancer' elements such as the MoMuSV 'enhancer'. This conclusion agrees with a preliminary report⁴⁰ that the 69% *Hind*III-*Bam*HI fragment 'enhances' TK transformation. More recently, using an SV40 target gene these workers have located an enhancer element in the right-hand end of fragment A (M. Lusky, personal communication).

The low efficiency of the BPV-1 DNA fragments in activating the *tk* gene in pTK10 relative to pTK1 is not understood, but may have several causes. The restriction endonuclease sites used for cloning may not be optimal for recovery of activity, or the results may reflect the low level of BPV-1 expression encountered in *in vitro* transformed cells⁵⁻⁷. Neither can we exclude the possibility that some of the observed activity is due to biologically irrelevant sequences, but several considerations suggest that this is not the case for all fragments. The four active fragments all map within the 69% *Hind*III-*Bam*HI fragment that contains the early gene sequences⁵⁻⁷, including the transformation gene(s)⁴ (Fig. 2). Fragments B' and C are located proximal to the 5' leaders of the early viral transcripts⁵. They both gave the highest number of transformed colonies when inserted into pTK10 in such a way that their orientation relative to the direction of transcription of *tk* and BPV-1 was the same. This behaviour is similar to that observed for non-enhancer 5' transcriptional control sequences of other well characterized eukaryotic genes such as the HSV *tk* gene promoter and the human ϵ -globin gene promoter. Both B' and C contain a TATAAA sequence (related to a consensus sequence found in most eukaryotic promoter regions), with a potential ACA cap site located approximately 30 bp downstream. A potential splice donor sequence is located 40 bp downstream from the TATAAA sequence in B', and 125 bp downstream from that in C⁸. In addition, a DNase I-sensitive region has recently been located within the B'-C region in BPV-1 chromatin isolated from transformed fibroblasts (G. Sauer, personal communication). DNase I sensitivity has been shown to correlate with active transcriptional control elements⁴¹. Taken together, these observations strongly suggest that fragments B' and C contain functionally active transcriptional control signals operative during the life cycle of the virus. Further experiments may determine whether either or both control expression of the early and late transcripts.

The ability of fragment A to activate the *tk* gene is shared with the MoMuSV enhancer element and is presumably related to their 'enhancing' properties. This, however, does not appear to be the case for fragment L. The position of the enhancer of BPV-1, relative to the other control sequences, is different from those previously described⁹⁻¹³, where the enhancer elements are located immediately adjacent to the 5' side of the promoter sequences. In contrast, the BPV-1 enhancer is 3-5 kb away from fragments B' and C, and is located 3' to fragment L. The functional significance of this novel arrangement is not yet understood.

The structure of the *tk* mRNAs present in the TK⁺ transformants is not known. Given the multiplicity of 5' ends identified in RNA transcribed from both strands of the HSV-1 *tk* sequence⁴², the pattern of transcription will probably be complex. By using S1 mapping in short-term expression assays, it has been found that the *tk* mRNAs of transformants induced by a pTK10 recombinant plasmid containing the mouse β major globin gene promoter⁴³, have heterogeneous 5' ends, which map both within and outside the *tk* gene (S. Gilmour and D.A.S., unpublished results). We expect the *tk* mRNA coded for by the pTKBV1 plasmids to have equally heterogeneous 5' ends.

We are currently analysing the chromosomal state of the pTKBV1 plasmids in the TK⁺ transformants. Preliminary results indicate that in all cases the plasmid DNA is present in the high molecular weight fraction of DNA, with the exception of the plasmids containing fragment B'. These are found in the low molecular weight fraction of cell DNA, thus warranting further investigation into their molecular structure.

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Novel renin inhibitors containing the amino acid statine

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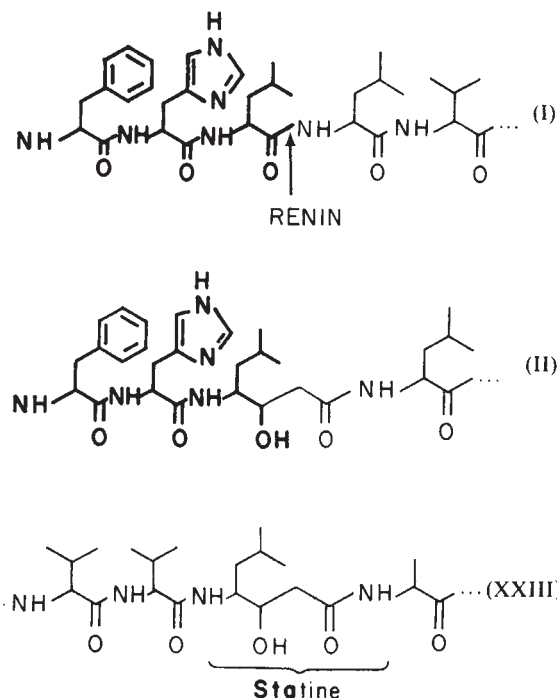


Fig. 1 Renin inhibitor sequence analogy. Portions of the structures of pig renin substrate I, the substrate-based renin inhibitor II, and the naturally occurring inhibitor pepstatin XXIII. Bold portions of I and II show areas where sequence analogy between substrate and inhibitor is apparent and confirmed by structure activity relationships. The lower potency of pepstatin XXIII as a renin inhibitor may be a consequence of poor sequence analogy to I in this region. The statine residue in II is thought to serve as an analogy of the transition state in the hydrolysis of I.

The proteolytic enzyme renin (EC3.4.99.19) cleaves the protein substrate angiotensinogen to yield angiotensin I, the decapeptide substrate transformed by converting enzyme into the pressor substance angiotensin II¹. Although the contribution of this pathway to the maintenance of normal blood pressure is unclear, it seems to be a major factor in various hypertensive states²⁻⁴. Important progress in the control of hypertension has been achieved by development of the potent inhibitors SQ-14,225 (captopril)^{5,6} and MK-421 (enalapril maleate)⁷⁻⁹ which block the generation of angiotensin II by the inhibition of angiotensin converting enzyme. An attractive alternative to the inhibition of converting enzyme would be the blockade of the preceding step in the cascade, the renin reaction. We report here new highly potent ($IC_{50}=10^{-9}$ – 10^{-8} M) competitive inhibitors of renin in which statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, is incorporated into analogues of the pig renin substrate (Fig. 1).

Competitive inhibitors of primate renin (for example compound XXIV in Table 2, $K_i=1$ – 25×10^{-6} M) were developed by Burton and coworkers by replacing one or both of the Leu residues flanking the cleavage site in pig angiotensinogen (6–13) octapeptide I (Table 1) with Phe residues^{10,11}. Szelke and colleagues obtained potent competitive inhibitors of canine renin ($IC_{50}=2$ – 3×10^{-8} M) by replacing the Leu 10–Leu 11 peptide bond in pig angiotensinogen (6–13) octapeptide I with a reduced isostere as in compound XXV (Table 2), that is $-\text{CH}_2-\text{NH}-$ replaces $-\text{CO}-\text{NH}-$ ¹². These same workers recently reported a similarly potent, selective inhibitor of human renin, in which the reduced isostere is incorporated into the analogous human substrate sequence and extended to a decapeptide (compound XXVI, Table 2)¹³. The general aspartyl-protease inhibitor pepstatin, isovaleryl-Val-Val-Sta-Ala-Sta (compound XXIII, Table 2), a naturally occurring pentapeptide containing two units of the unusual amino acid statine (Sta), also inhibits renin and other acid proteases^{14,15}. It has been proposed that the 3*S*-hydroxyl of this internal Sta residue is an analogue of the transition-state or tetrahedral-intermediate for the enzyme reaction, thereby enabling the efficient binding to renin, even though pepstatin bears no close structural relationship to the minimal renin substrate sequence I¹⁶.

While pepstatin is indeed a potent inhibitor of renin (pig kidney) with $K_i=3.9 \times 10^{-7}$ M at pH 7.3 (see below), it is much more effective against other aspartyl proteases, for example K_i for pepsin is 4.6×10^{-11} M¹⁷. Considering the extraordinary substrate specificity exhibited by renin, for which the minimum kinetically-competent substrate is an octapeptide portion (6–13) around the cleavage site (compound I, Fig. 1)¹⁸, we postulated that incorporation of statine into a peptide sequence more closely resembling that of angiotensinogen would yield improved inhibitors of renin.

Several analogues of renin substrate (6–13) were prepared to explore the effects of statine position and peptide chain

length on inhibitory potency. It was assumed that the 3*S* hydroxyl group of statine mimicked some intermediate in the hydrolysis of the amide bond between Leu 10 and Leu 11 and that the isobutyl side chain of statine could be isosteric with either Leu side chain. Furthermore the rate-enhancing effects of peptide substituents at points distal to the peptide cleavage site had been demonstrated previously for both pepsin¹⁹ and renin¹⁸ so that peptide chain-length was expected to influence inhibitor potency if the statine 3*S*-hydroxyl group formed a mechanistically derived complex with renin.

Table 1 lists some of the synthetic analogues used to determine the minimal sequence for potent binding to renin. All peptides were prepared either in solution or by solid phase syntheses. Analogues were fully characterized by ¹H nuclear magnetic resonance, microanalysis, amino acid analysis, thin-layer chromatography and high pressure liquid chromatography. Details of these syntheses will be reported elsewhere.

Our initial hypothesis was that Sta could replace Leu 10 of the substrate sequence and might function as a dipeptide analogue of Leu 10–Leu 11, due to its two extra backbone atoms as compared to an α -amino acid. Heptapeptide II (Table 1) inhibits pig kidney renin with an IC_{50} more than 1,000-fold lower than the K_M value for the comparable octapeptide substrate I. Amino-terminal acylation gives inhibitors of comparable potency (compounds III, IV, XI) with possible increased enzymatic stability (aminopeptidase resistance). Extension from the minimally inhibitory tripeptide V to the full heptapeptide, for example V $\rightarrow$ VI $\rightarrow$ VII $\rightarrow$ VIII $\rightarrow$ II, establishes the importance of renin substrate derived substituents in positions 6–9, with a major increase in inhibition brought on by the Phe 8 residue (VI $\rightarrow$ VII). Stepwise shortening of the C-terminal portion of compound XI, as in X $\rightarrow$ IX, establishes the importance of the two amino acid residues extending from the statine carboxyl. Further extension of the C-terminal peptide chain does not increase potency. Methyl esters, for example

Table 1 Inhibition of pig kidney renin by statine-containing substrate analogues

Compound no.	Structure								IC ₅₀ (M)
	6	7	8	9	10	11	12	13	
I	His-Pro-Phe-His-Leu-Leu-Val-Tyr								(5.5 × 10 ⁻⁵)*
II	His-Pro-Phe-His-Sta—Leu—Phe-NH ₂								2.0 × 10 ⁻⁸
III	Iva-His-Pro-Phe-His-Sta—Leu—Phe-NH ₂								3.1 × 10 ⁻⁸ †
IV	Boc-His-Pro-Phe-His-Sta—Leu—Phe-NH ₂								2.7 × 10 ⁻⁸
V	Sta—Leu—Phe-NH ₂								2.0 × 10 ⁻³
VI	His-Sta—Leu—Phe-NH ₂								3.7 × 10 ⁻⁴
VII	Phe-His-Sta—Leu—Phe-NH ₂								1.3 × 10 ⁻⁶
VIII	Pro-Phe-His-Sta—Leu—Phe-NH ₂								2.0 × 10 ⁻⁷
IX	Ibu-His-Pro-Phe-His-Sta-NH ₂								2.9 × 10 ⁻⁵
X	Ibu-His-Pro-Phe-His-Sta—Leu-NH ₂								6.7 × 10 ⁻⁷
XI	Ibu-His-Pro-Phe-His-Sta—Leu—Phe-NH ₂								4.3 × 10 ⁻⁸
XII	Ibu-His-Pro-Phe-His-Sta—Ala—Phe-NH ₂								5.7 × 10 ⁻⁸
XIII	Ibu-His-Pro-Phe-His-Sta—Val—Phe-NH ₂								1.2 × 10 ⁻⁷
XIV	Iva-His-Pro-Phe-His-Sta—Ile—Phe-NH ₂								1.3 × 10 ⁻⁷
XV	Boc-His-Pro-Phe-His-Sta—Tyr-NH ₂								2.6 × 10 ⁻⁸
XVI	Boc-His-Pro-Phe-His-Sta—Leu—Phe-OCH ₃								1.1 × 10 ⁻⁸
XVII	Iva-His-Pro-Phe-His-Sta-Leu-Val-Phe-NH ₂								4.6 × 10 ⁻⁸
XVIII	POA-Leu-Sta—Val—Phe-OCH ₃								4.5 × 10 ⁻⁶
XIX	POA-Leu-Sta—Leu—Phe-OCH ₃								4.5 × 10 ⁻⁶
XX	POA-His-Sta—Leu—Phe-OCH ₃								2.9 × 10 ⁻⁶
XXI	Iva-His-Pro-Phe-His-Leu-Sta-Val-Phe-NH ₂								1.5 × 10 ⁻⁴
XXII	Iva-His-Pro-Phe-His-Sta‡—Leu—Phe-NH ₂								1.4 × 10 ⁻⁵
	(3R)								

Pig kidney renin was assayed as previously described²², using a ¹⁴C-Leu labelled decapeptide substrate, except the pH was raised to pH 7.3 by use of 0.05 M citrate/phosphate buffer, 30 °C. Enzyme concentration was determined to be 9 × 10⁻⁹ M, substrate concentration was 8.0 × 10⁻⁵ M, and substrate K_M = 5 × 10⁻⁵ M. IC₅₀ values (concentration for 50% inhibition) were determined by linear regression of logit against log concentration over a 15–90% inhibition range, using 3–6 concentrations. IC₅₀ values were found to be routinely reproducible within ±20%. Compounds were synthesized by standard peptide methods either by solid-phase techniques according to Merrifield²⁴ or by solution phase methods similar to those previously described²². The synthesis of suitably protected statine has been described²⁵. Compounds were characterized by TLC (silica gel), HPLC (reversed phase), ¹H NMR, amino acid analyses, mp and elemental analyses. Details of syntheses and characterization will be reported separately. Abbreviations: Iva, isovaleryl (3-methylbutyryl); Ibu, isobutyryl (2-methylpropanoyl); Boc, *tert*-butoxycarbonyl; Sta, statyl ((3*S*, 4*S*)-4-amino-3-hydroxy-5-methyl-heptanoyl); POA, phenoxyacetyl. The numbering scheme above the compound list is that of the pig renin substrate, the minimum kinetically competent fragment of which is I, and reflects the hypothesized analogy between inhibitors and substrate.

* K_M value¹⁸.

† 3.10 ± 0.37 × 10⁻⁸ M (mean ± s.e. of 4 experiments using three different synthetic samples and assays on different days).

‡ (3*R*, 4*S*)-4-amino-3-hydroxy-5-methyl heptanoyl, the Sta isomer epimeric at the 3-hydroxyl.

Table 2 Species specificity of renin inhibitors

Compound no.	Structure	Renin, IC ₅₀ , M				
		Human plasma	Human kidney	Dog plasma	Pig kidney	Rat plasma
III*	Iva-His-Pro-Phe-His-Sta—Leu—Phe-NH ₂	1.6 × 10 ⁻⁸	1.9 × 10 ⁻⁸ †	4.2 × 10 ⁻⁸	3.1 × 10 ⁻⁸	6.0 × 10 ⁻⁷
XIV	Iva-His-Pro-Phe-His-Sta—Ile—Phe-NH ₂	1.9 × 10 ⁻⁹	1.9 × 10 ⁻⁹ †	3.8 × 10 ⁻⁸	1.3 × 10 ⁻⁷	7.1 × 10 ⁻⁷
XXIII	Iva-Val-Val-Sta—Ala—Sta	2.2 × 10 ⁻⁵	1.3 × 10 ⁻⁵ †	1.3 × 10 ⁻⁶	1.0 × 10 ⁻⁶	2.6 × 10 ⁻⁵
XXIV§	Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr	7.0 × 10 ⁻⁶ ‡	1.3 × 10 ⁻⁶ †	7.0 × 10 ⁻⁵	3.0 × 10 ⁻⁵	7.0 × 10 ⁻⁵
XXV	D-His-Pro-Phe-His-Leu ^R Leu-Val-Tyr	1.0 × 10 ⁻⁶	NR	2.4 × 10 ⁻⁸	NR	6.3 × 10 ⁻⁷
XXVI¶	Pro-His-Pro-Phe-His-Leu ^R Val-Ile-His-Lys	1.0 × 10 ⁻⁸	1.0 × 10 ⁻⁸	1.0 × 10 ⁻⁵	NR	NR

For compounds III, XIV and XXIII: Pig kidney renin was assayed as described in Table 1. Human, dog, rat, and other plasma renin assays were performed by radioimmunoassay for angiotensin I as described by Haber *et al.*²⁶ using a commercial kit (Clinical Assays), at pH 7.4 (phosphate, 37 °C). The optimum angiotensinase inhibitor for assays at pH 7.4 was found to be 8-hydroxyquinoline, superior to phenylmethylsulphonyl fluoride (PMSF) in rat plasma²⁷ and in dog plasma, and equal to PMSF in human plasma at this pH. Plasma samples (pooled) were collected on ice in EDTA from mongrel dogs, Sprague-Dawley rats, cebus monkeys, and cats. High plasma renin samples were obtained when required from animals pretreated with furosemide, hydrochlorothiazide, or by maintenance on a low-sodium diet. Lyophilized human plasma was obtained from Ortho Diagnostics (low renin) or Clinical Assays (high renin). IC₅₀ values were obtained as described²⁸ and should be considered to be reproducible to ±30%. The 95% confidence limits obtained by data analysis of a single experiment were routinely ±10%. Human kidney renin assay was performed with synthetic tetradecapeptide renin substrate by a fluorometric method²⁸, pH 7.20 (0.10 M, citrate/phosphate), 37 °C. Human kidney renin was International Standard human kidney renin²⁹ that was further purified by affinity chromatography on pepstatin-aminohexyl-Sepharose²⁸. Inhibition data were consistent with competitive inhibition, and K_i values were determined from linear I/V versus I/S plots²³, with an estimated error of ±20%. Inhibition data for compounds XXIV, XXV and XXVI were obtained from refs 11, 12 and 13, respectively. Assays for these compounds were performed in similar conditions to those described above, but data comparisons should be made cautiously. NR, not reported. For abbreviations see Table 1. Additionally, 'Leu^RLeu' or 'Leu^RVal' indicates a reduced isosteric bond, that is —CH₂NH— in place of the peptide bond —CO—NH—.

* The following additional IC₅₀ values were obtained for plasma renin inhibition at pH 6.0 (maleate buffer): human, 1.9 × 10⁻⁸ M; cebus monkey, 5.3 × 10⁻⁹ M; mongrel dog, 1.8 × 10⁻⁸ M; beagle dog, 1.3 × 10⁻⁸ M; cat, 4.1 × 10⁻⁸ M; and Sprague-Dawley rat, 1.3 × 10⁻⁷ M.

† K_i value, M.

‡ Estimated from data in ref. 11: 30% inhibition at 4 × 10⁻⁶ M; 60% inhibition at 1 × 10⁻⁵ M.

§ Ref. 11.

|| Ref. 12.

¶ Ref. 13.

Table 3 Acid protease specificity of renin inhibitors

Compound no.	Structure	$K_i, 10^{-9}$ M			
		Human renin	Pig renin	Cathepsin D	Pepsin
XIV	Iva-His-Pro-Phe-His-Sta-Ile-Phe-NH ₂	1.9	50	134	43
III	Iva-His-Pro-Phe-His-Sta-Leu-Phe-NH ₂	19	10	210	27
II	His-Pro-Phe-His-Sta-Leu-Phe-NH ₂	50	6	900	40
XX	POA-His-Sta-Leu-Phe-OCH ₃	6,300	1,100	3,300	67
XIX	POA-Leu-Sta-Leu-Phe-OCH ₃	27,000	1,700	35	12
XXIII	Iva-Val-Val-Sta-Ala-Sta	13,000	390	0.5*	0.05†

Human kidney renin inhibition constants (K_i) were determined as described in Table 2. Pig kidney renin IC_{50} values were determined as described in Table 1, and K_i values were calculated, assuming competitive inhibition, using the equation of Cha³⁰: $K_i = (IC_{50} - E_t/2)(1 + S_0/K_M)^{-1}$, where $E_t = 9 \times 10^{-9}$ M (enzyme concentration), $S_0 = 8.0 \times 10^{-5}$ M (substrate concentration), and $K_M = 5 \times 10^{-5}$ M. Rabbit liver cathepsin D inhibition constants were determined as previously described³¹, using [¹⁴C-methyl]glycylated bovine haemoglobin substrate, pH 4.0 (citrate buffer), with data analysis by Dixon plots²³, giving K_i values. Porcine pepsin (Sigma) was assayed at pH 4.0 (formate buffer) using a heptapeptide substrate Phe-Gly-His-Phe(4-NO₂)-Phe-Ala-Phe-OCH₃ followed at 310 nm³². Using several inhibitor concentrations, IC_{50} values were calculated as for pig kidney renin assay (Table 1). Using the Cha equation³⁰, as described above, K_i values could be calculated using the values: $E_t = 1.2 \times 10^{-8}$ M; $S_0 = 1.0 \times 10^{-4}$ M; and $K_M = 1.25 \times 10^{-5}$ M. Abbreviations: see Table 1.

* Value from ref. 33. The measured value in the assay used here is 2.5×10^{-9} M, likely an overestimation due to tight-binding of the inhibitor and enzyme depletion³⁴.

† Value from ref. 17. The measured value of $IC_{50} = 5.9 \times 10^{-9}$ M is the enzyme concentration limiting value ($E_t/2$).

compound XVI, while less soluble, are slightly better inhibitors than C-terminal amides.

The analogy between substrate I and inhibitors IX–XVII with respect to substrate positions 11 to 13 remains unclear. The inhibition data establish that addition of Leu and Phe to Sta 10 increases binding 700-fold, but it is uncertain whether these amino acids are analogous to positions 11–12 or 12–13 in renin substrate, or bind in other positions not exactly analogous to substrate. Statine can be considered to be a transition-state analogue replacing only Leu 10 (as in XVII) or as a dipeptide analogue of the transition-state complex replacing both Leu 10–Leu 11. The data presented here do not distinguish between these models. The single Leu 10 replacement hypothesis cannot be exact, since XVII is no better an inhibitor than III. The dipeptide analogy must also be imprecise since XIII is a weaker inhibitor than XI. Since position 13 is known to be required for substrate binding¹⁸, we tentatively propose that the terminal Phe in inhibitors related to III, IV and XI is bound in a position analogous to substrate position 13, and that the residue following Sta roughly corresponds to position 12. Molecular modelling studies and human substrate analogues provide additional evidence for this approximate dipeptide analogy hypothesis and will be reported separately. A similar analogy between pepstatin and pepsin substrates has been proposed based upon analysis of pepsin subsite specificity²⁰.

Tetrapeptides XVIII and XIX were prepared to test the possibility that Sta might replace Leu 11 rather than Leu 10 as in the inhibitors discussed above. These tetrapeptide analogs are now recognized to be analogous to substrate residues 8–13 (in I) with Sta replacing Leu 10, as in XX. Indeed, incorporation of Sta as a replacement for Leu 11 in the full octapeptide inhibitor XXI gives a very poor inhibitor.

The identity of the renin substrate and inhibitor structures in positions 6–10 and their comparable effects on binding to renin provide strong evidence that these inhibitors act in a mechanistically related fashion. As the substrate positions 9 to 6 are filled with amino acids found at these sites in angiotensinogens, the tetrapeptide Leu-Leu-Val-Tyr (or Phe) is converted from a weak inhibitor ($K_i = 3 \times 10^{-4}$ M) to the good substrate I ($K_M = 5.5 \times 10^{-5}$). In the inhibitor series these structural changes cause a 10,000-fold decrease in dissociation constant as V is extended to II ($K_i = 6 \times 10^{-9}$ M for II assuming competitive inhibition). Furthermore when the chirality of the 3-hydroxyl group transition-state feature in statine is reversed (from 3S to 3R), as in heptapeptide XXII, there is a 500-fold loss in inhibition. The net gain in binding energy of II, relative to substrate I or inhibitor XXII, is greater than 4–5 kcal. This remarkable increase in binding is of the order of magnitude expected for a transition-state analogue²¹.

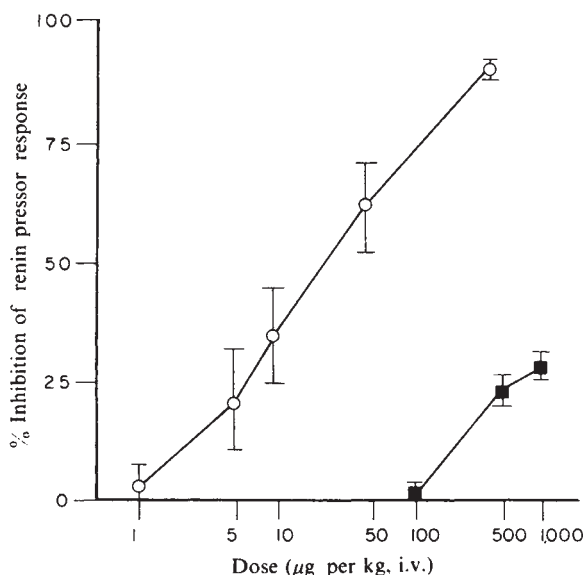


Fig. 2 Inhibition of pig kidney renin pressor response in anaesthetized rats by Boc-His-Pro-Phe-His-Sta-Leu-Phe-NH₂ (IV, ○) and by pepstatin A, Iva-Val-Val-Sta-Ala-Sta (XXIII, ■). The ability of compounds IV and XXIII to inhibit the pressor response to renin was determined in mecamylamine (1.25 mg per kg i.v.)—pretreated anaesthetized (Dial Urethane, 1 ml per kg i.p.) male rats (CRCDI strain). After mecamylamine treatment, mean blood pressure (carotid artery) fell from about 120 mm Hg to about 50 mm Hg. After this pressure stabilized, hog kidney renin (same preparation used for *in vitro* inhibition as described in Table 1), dissolved in isotonic saline, was infused (right jugular vein) at 0.11 GU per min (0.11 ml per min) for 5 min, followed by a sustaining infusion of 0.028 GU per min. This sustaining infusion maintained blood pressure (bp) at about 120 mm Hg. Injection of IV, in saline containing 0.1% acetic acid, into the left femoral vein caused a rapid (<30 s), dose-dependent fall in bp towards the pre-renin-infusion level, followed by a recovery phase back to the renin-maintained level. Injections were spaced at least 20 min apart, or longer for higher doses, to allow for full recovery. Vehicle control injections caused no significant effects. Per cent inhibition (100% = pre-renin-infusion level, about 50 mm Hg; 0% = renin maintenance level, about 120 mm Hg) is plotted against dose. A dose of 2 mg per kg of IV causes ~100% inhibition which requires more than 90 min for recovery. Inhibition caused by pepstatin XXIII, administered as the Na salt in distilled water, causes only ~25% inhibition at the highest dose tested, 1 mg per kg, limited by solubility. $ID_{50} = 0.030$ mg per kg for IV, five rats at each dose (0.019–0.054 mg per kg, 95% confidence limits). $ID_{50} > 1.0$ mg per kg for XXIII, four rats at each dose.

A comparison of relative inhibitions of the most potent known renin inhibitors with that of III against renins from human, dog, pig and rat is shown in Table 2. Only the reduced isosteric bond analogues XXV and XXVI show activity comparable to III. The former¹² is specific for canine renin, and the latter¹³ for human renin. Our inhibitor XIV is the most potent inhibitor of human renin yet reported, and it retains high potency versus canine renin.

Analogues III and XIV are substantially better inhibitors of renin than of either pepsin or cathepsin D as shown in Table 3 and are very weak inhibitors of human converting enzyme, human leukocyte elastase, porcine pancreatic elastase, bovine trypsin, bovine α -chymotrypsin, or porcine kidney leucine aminopeptidase at 5×10^{-5} M (data not shown).

The antihypertensive activity of renin inhibitor IV was evaluated in a ganglion-blocked anaesthetized rat model, in which the blood pressure is maintained (at ~ 120 mm Hg) by a continuous infusion of pig kidney renin. In this model, pepstatin XXIII, administered as a bolus intravenously, has an ID₅₀ of >1 mg per kg, where ID₅₀ is defined as that quantity of compound required to lower the measured blood pressure by 50% toward the pre-renin-infusion level (typically, 50 mm Hg). The ID₅₀ for IV is 0.030 mg per kg, more than 50 times more potent on a molar basis (see Fig. 2). When blood pressure was maintained by angiotensin I, angiotensin II, or noradrenaline, neither IV nor XXIII lowered blood pressure, consistent with the antihypertensive action being due to inhibition of renin. The duration of action of the ID₅₀ dose, defined as the time from half-maximal effect to half-recovery, is approximately 15 min for IV compared to <2 min for pepstatin XXIII. Larger doses of IV give a flat return to pre-renin-infusion levels of blood pressure, with a 2 mg per kg dose having a duration of almost 90 min. Further biological characterization of the mode of action of these inhibitors is in progress.

The renin inhibitors described above are the most potent such compounds yet reported. While problems remain to be solved before medically useful agents result, these inhibitors possess already the inherent potency characteristic of medically useful compounds. Further study of the inhibition of renin *in vivo* using the compounds reported here should serve to elucidate the contribution of the renin system to various disease states. The design of these renin inhibitors, using abstraction of a transition-state feature from an inhibitor of a related enzyme and mechanistically related incorporation of this feature into the substrate sequence, suggests a general approach to the design of protease inhibitors.

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Evidence for a conservative pathway of transposition of bacteriophage Mu

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During its lytic cycle bacteriophage Mu uses repeated transposition as a mode of DNA synthesis. These transpositional events are undoubtedly replicative, and presumably semi-conservative. In a Mu lysogen this type of transposition can start immediately after prophage induction. However, in an infective cycle the Mu genome (which is injected into the host cell as a linear molecule flanked by short random sequences of bacterial DNA) must first become integrated into the host chromosome. Little is known about how this occurs apart from the fact that the bacterial sequences at either end of the Mu genome are lost in the process¹. The integration is thus similar to a transposition event. In an attempt to determine whether this type of Mu transposition (between a linear donor molecule and a circular recipient) is also semi-conservative we have analysed the progeny phage arising from an infective cycle in which the parental DNA was heterozygous for a known genetic marker. The expectation is that if integration of the infecting Mu genome occurs by a single semi-conservative transpositional event then pure phage bursts should be produced as the genetic information on only one strand would be preserved throughout the lytic cycle. The experiments reported here do not support this expectation in that the infected cells yield mixed bursts, suggesting that Mu integration is a conservative, rather than a semi-conservative event.

To ensure that the infecting DNA was heterozygous we used transfection rather than phage adsorption as a starting point in the investigation. This allowed the heterozygous molecules to be prepared by denaturation and reannealing, a device used previously in analogous studies with *Bacillus subtilis*² phages and with phage λ (ref. 3). As it has already been reported that Mu DNA derived from phage particles and treated in this way is not active in transfection⁴ (presumably because of the presence at either end of single-stranded 'split-ends' of bacterial DNA susceptible to degradation by host nucleases) we isolated our linear Mu DNA from the plasmid pSU1 (Fig. 1). This contains an insertion of the Mu derivative MupApl into the ColE1-type plasmid pML2 (ref. 5). It can be seen from Fig. 1 that all the *Sma*I sites in pSU1 are in the pML2 moiety, complete restriction with this endonuclease yielding a linear molecule containing the Mu genome flanked now by two unique sequences of pML2 DNA amounting to about 0.05 kilobases (kb) at the *c* end of Mu, and 9.0 kb at the β end.

We isolated linear Mu DNA in this way from pSU1, and first compared its ability to transfect competent cells with that of DNA obtained from phage particles by phenol extraction. Both preparations transfected with equal efficiency. This result is of some interest, as although the foreign DNA at the *c* end of Mu is of a comparable length in both samples (0.05 kb), the

Table 1 Summary of transfection results

Source of Mu DNA*	Transfection frequency†
Phenol extraction from phage particles	3.0×10^3
Excision from pSU1 with <i>Sma</i> I	3.0×10^3
Excision from pSU1 with <i>Sma</i> I followed by denaturation and reannealing	2.0×10^2 – 2.0×10^3

Transfection experiments were carried out using the *recBC* Su^+ strain PP244 which harbours a defective MuCts prophage. This prophage is induced before transfection to allow the expression of an early Mu gene necessary for successful transfection. The procedure used was that originally developed and described by van de Putte *et al.*⁸. Heat denaturation and reannealing were performed according to procedures described previously³.

* Experiments were performed using both B^+ and B^- DNA.

† All transfection frequencies are expressed as numbers of plaques per μ g of MuApl DNA. Optimal transfection for all sources of Mu DNA occurs at a final concentration of 25μ g ml⁻¹.

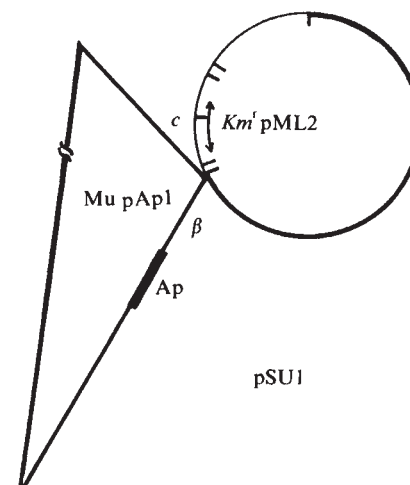


Fig. 1 Position of *Sma*I restriction sites in pSU1. As shown, all six sites are in pML2. The thick line represents the molecule active in transfection after complete restriction with *Sma*I. It consists of 36 kb of Mu DNA and ~9 kb of pML2.

foreign DNA at the β end is 7 kb longer in the molecules derived from pSU1. We also tested the ability of linear DNA from pSU1 to transfect after the strands had been separated and reannealed. This ability was found to vary over roughly a 10-fold range depending in some unknown way on the conditions of strand separation and reannealing, the highest values being almost identical to those obtained with the native DNA. As well as extracting DNA from pSU1 we also prepared linear molecules from a derivative pSU1 B^- in which an amber mutation had been introduced into the MuB gene of pSU1 by genetic recombination⁶. When used to transfect competent cells this DNA had the same characteristics as that derived from pSU1. These preliminary results are summarized in Table 1.

Having shown that the reannealed DNA derived from pSU1 had transfecting ability it was then possible to perform the heterozygote experiment. Linear MuDNA obtained from pSU1 and pSU1 B^- was mixed in equimolar amounts, heat-denatured and the strands allowed to reanneal at random. This reannealed DNA, which in theory consists of 50% B^+/B^- heterozygotes and 25% each of B^+/B^+ and B^-/B^- homozygotes, was used to transfect competent cells that were plated on a Su^+ indicator before lysis. Individual plaques were then picked into broth and plated at 37°C on a mixed indicator of Su^+ and Su^- bacteria, on which it was easy to distinguish wild type from *amB* plaques as the latter were smaller and more turbid. In a preliminary experiment of this type, almost 50% of the transfected cells yielded a mixture of wild type and *amB* phage. As this result could have arisen from multiple infection of competent cells, however, two controls were introduced into the experiment. The first consisted of a series of transfections using decreasing concentrations of DNA; the second in performing a parallel series of transfections with linear DNA derived from pSU1 and pSU1 B^- , in which the strands had been separated and reannealed before being mixed in equimolar amounts. Results of a typical experiment incorporating these controls are shown in Table 2. It can be seen that decreasing the concentration of heteroduplex DNA by a factor of eight had no systematic effect on the fraction of competent cells yielding both wild type

and *amB* phage, which remained close to 50%. Some mixed bursts were also observed in the homozygous control experiments, but the fraction decreased with decreasing DNA concentration and even at the highest level this value (14%) was too low to have a significant effect on the heteroduplex results. It was further observed that almost all mixed bursts contained approximately equal numbers of wild type and *amB* phages.

Although our experiment was performed using transfection, the results can reasonably be expected to hold after phage adsorption. The clear conclusion from the experiment is that the information on both strands of the infecting Mu DNA is transmitted with equal efficiency to progeny phage. It is possible to reconcile this result with a semi-conservative mechanism of transposition, either by postulating that at least two successive integration events occur after Mu infection, or that integration occurs by a 'cointegrate formation' mode. However there are problems with both these interpretations, particularly in relation to phage infections that lead to lysogeny, as by the first explanation multiple lysogens should usually be formed (while in fact mono-lysogens are the rule), while the second implies linearization of the host chromosome, which would lead inevitably to cell death. The most straightforward interpretation of our experiment is that the initial transposition by which the Mu genome becomes integrated is a single conservative event. Independent evidence of quite a different kind which supports this idea comes from recent work of Liebart *et al.*⁷. In these experiments, isotope labels were used to follow the fate of Mu phage DNA after adsorption to cells harbouring the plasmid pBR322. Soon after infection, supercoiled plasmid DNA was isolated and further analysis suggested that some of this DNA consisted of pBR322 molecules into which Mu had integrated conservatively.

If it is accepted that Mu can transpose conservatively as well as semi-conservatively, then obvious questions that arise are what factors determine the particular pathway of transposition

Table 2 Analysis of phage bursts from competent cells transfected with linear pSU1 and pSU1 B^- DNA

DNA concentration μ g ml ⁻¹	Bursts from DNA mixed before denaturation and reannealing				Bursts from DNA mixed after denaturation and reannealing			
	Wild type and <i>amB</i>	Pure wild type	Pure <i>amB</i>	% Heterozygotes	Wild type and <i>amB</i>	Pure wild type	Pure <i>amB</i>	% Heterozygotes
25	68	33	29	48	14	46	40	14
15	50	28	27	52				
12	53	26	21	47				
8	37	18	15	53	7	29	23	9
3	16	8	7	52	2	17	15	6

to be followed, and whether both modes of transposition are open to other transposons. One possibility consistent with the Mu results is that when the donor molecule is supercoiled, transposition is semi-conservative, otherwise it is conservative.

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Structure of trimeric haemerythrin

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Several simplifying structural principles have been developed from the considerable data contained in the three-dimensional structures of proteins determined in the past two decades. One of these is based on the observation that particular folding motifs often occur in a variety of structural and functional settings¹. The compact bundle of four antiparallel α -helices², first seen in the structure of myohaemerythrin³, is an example. Several non-haemerythrin proteins have since been found to have the same folding pattern^{4–7}, and haemerythrins themselves exist in a wide variety of quaternary arrangements^{8,9}. The unusual ability of the haemerythrin fold to associate as dimers, trimers, tetramers, octamers or higher aggregates provides an opportunity for examining structural diversity in subunit association. We have used X-ray crystallography to study the subunit structure of trimeric haemerythrin from a *Siphonossoma* species. We report here that the pattern of intersubunit helix-helix interactions differs from the most common mode of association of other helix-bundle proteins. In a novel approach to structure analysis at low resolution, experimental phases for the structure determination were based on anomalous scattering from the iron atoms native to haemerythrin, using the new resolved-anomalous phasing procedure¹⁰.

Haemerythrins are oxygen transport proteins found in the coelom, vascular system and musculature of several invertebrates, most notably the Sipuncula, a phylum of marine worms. They are one of three classes of oxygen transport proteins that have been characterized: haemoglobins, with one porphyrin-bound Fe atom per subunit; haemerythrins, with two Fe atoms bound by protein side chains; and haemocyanins, with two Cu atoms. These represent different chemical and structural solutions to the biological problem of reversible oxygenation. Each haemerythrin molecule has a specific aggregation state, and although earlier studies suggested association only as octamers, recent work has shown that trimers are quite common and widely distributed^{9,11,12}. Crystal structures of a myohaemerythrin monomer³ and of two coelomic haemerythrin octamers^{13,14} have been reported previously. The amino acid sequences of these three proteins are known^{15–17}, but only composition data for the *Siphonossoma* trimer, and composition or incomplete sequence data for a few other haemerythrins are available.

Relative to the myohaemerythrin monomer, the two octamers have a five-residue deletion in one of the interhelix loops; overall the sequences are quite homologous¹⁷. A subunit of four antiparallel α -helices with an 18 residue N-terminal arm is common to these three haemerythrin structures, and all the available evidence indicates essentially the same subunit structure in other haemerythrins. The subunit molecular weight is approximately 14,000.

Erythrocyte haemerythrin was isolated from coelomic fluid of live specimens of *Siphonossoma* collected in mangrove swamps of Fiji, and the azidomet form of the protein was prepared¹² (the species identification in ref. 12 has been corrected⁹ by S. J. Edmunds (personal communication) to a *Siphonossoma* species near *Siphonossoma funafuti*). Further purification by isoelectric focusing was necessary to obtain large single crystals¹⁸ which were grown by vapour diffusion using polyethylene glycol as a precipitant. The crystals are monoclinic, belonging to space group P2₁, and have either cell constants $a = 80.31 \text{ \AA}$, $b = 45.11 \text{ \AA}$, $c = 63.58 \text{ \AA}$ and $\beta = 104.8^\circ$. The asymmetric unit of the crystal contains one trimeric molecule. Diffractometer data were measured to 4.5- $\text{\AA}$ spacings from one crystal using peak-top omega step-scans; Friedel pairs for acentric reflections were recorded to 5.5- $\text{\AA}$ spacings. The data were reduced by fitting gaussian distributions to peak profiles¹⁹ after subtraction of a smoothly varying background. Anomalous differences were calculated after application of anisotropic local scale factors²⁰.

Structure determination was based on anomalous scattering from the three di-iron centres in the trimer. The iron centres were located in an anomalous-difference Patterson synthesis²¹. This established the orientation and origin of the molecular 3-fold axis; the orientation of the axis was confirmed by calculation of a self-rotation function²² (the cross-rotation function, however, did not reveal the subunit orientations when isolated myohaemerythrin was used as a test structure). Experimental phases were calculated by the resolved-anomalous phasing procedure^{10,20} using the iron partial structure to resolve the phase ambiguity. Electron density maps were calculated from phase sets based on both crystallographic enantiomers of the Fe atom structure and the correct hand was chosen by visual examination of maps averaged about their respective local 3-fold axes. The haemerythrin fold was apparent even at this stage on careful inspection of the correct map. The quality of this map was improved, as in the case of octameric haemerythrin¹³, by further molecular averaging and by solvent levelling. The molecular envelope was determined by a semi-automatic procedure. During the early cycles of this phase refinement, calculated phases were combined with experimental phases²³ and the position of the molecular 3-fold axis was optimized by a least-squares procedure²⁴.

The phasing power of anomalous dispersion combined with molecular redundancy is illustrated by the clear images of the haemerythrin fold in the refined 'iron-resolved' electron density map—derived directly from the native data without recourse to isomorphous replacement or molecular repositioning. Appreciable errors due to the weak phasing ability of the iron partial structure are, however, observed in the iron-resolved map. These were eliminated by constructing an electron density map from properly orientated, isolated myohaemerythrin density functions, and calculating the corresponding structure amplitudes and phases by Fourier inversion (the amplitudes agreed with those observed at an R index of 0.336 for all data to 5.5 $\text{\AA}$ resolution). The calculated phases were used instead of those from the iron partial structure to resolve the ambiguities from anomalous scattering, and to produce, after a few cycles of phase refinement, the 'model-resolved' electron density map that was interpreted. Details of the experiment and the structure analysis will be published elsewhere, and atomic coordinates will be deposited in the Protein Data Bank²⁵.

Both iron-resolved and model-resolved electron density maps show conclusively that this coelomic haemerythrin trimer does not have the five-residue deletion relative to myo-

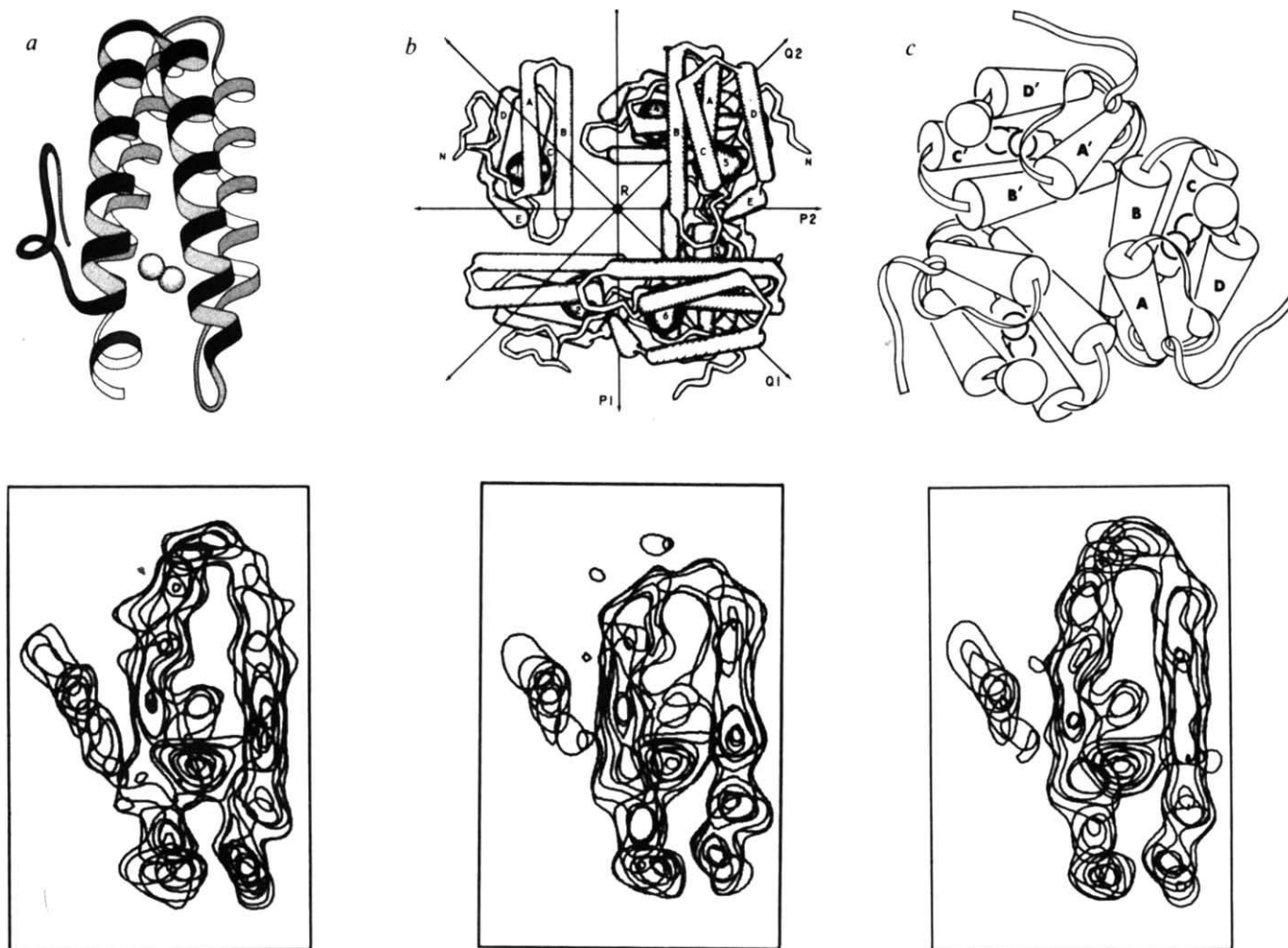
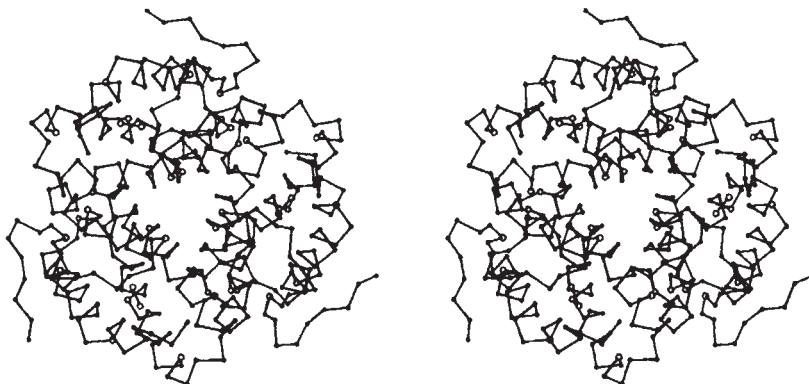


Fig. 1 Comparison of electron densities for the C and D helices of three haemerythrins: *a*, myohaemerythrin; *b*, octameric haemerythrin; and *c*, trimeric haemerythrin. From the right, each density frame shows the C helix running up, the D helix running down and a portion of the N-terminal arm. The five-residue deletion between the C and D helices of the octamer is readily apparent. The dense mass between the helices is the di-iron centre, and the density above the irons protruding from the D helix is the side-chain of Trp 102 (myohaemerythrin numbering). All maps were calculated at 5.5 Å resolution and are contoured starting from 0.53e Å⁻³ for *T. zostericola* myohaemerythrin³, 0.51e Å⁻³ for *P. gouldii* octameric haemerythrin¹³ and 0.52e Å⁻³ for trimeric haemerythrin, with successive contours at intervals of 0.2e Å⁻³. The map of myohaemerythrin was determined by multiple isomorphous replacement. The octamer map shown here was generated by molecular repositioning of myohaemerythrin electron density followed by four cycles of non-crystallographic symmetry averaging and solvent levelling. For the trimer, we show the model-resolved map described in the text.

Fig. 2 Stereo diagram of the C^α backbone of *Siphonosoma* trimeric haemerythrin. Coordinates are based on molecular repositioning of the refined atomic model of myohaemerythrin (unpublished). The view is along the molecular 3-fold axis. The subunit consists of an N-terminal arm of 18 residues on the outside of the trimer followed by an antiparallel helix bundle and a reverse turn at the C-terminus. The B helix is most involved in intersubunit contacts, while the D helix is external in the trimeric aggregate. Every tenth C^α atom and the iron atoms are highlighted by open circles.



haemerythrin that exists in the two known coelomic haemerythrin octamers. The deletion occurs in the loop between the C and D helices, and the corresponding absence of electron density is striking in octamer density functions (Fig. 1). A detailed comparison of model-resolved and myohaemerythrin electron density maps on an interactive graphics display shows that the subunit structures of the trimer and myohaemerythrin

are very similar and that the few small structural differences are confined to non-helical segments of the backbone. However, on the periphery of the subunit, where intramolecular contacts are made in the trimer, more significant differences are seen. There are a few new peaks of density in the trimer map that we tentatively interpret as bulky hydrocarbon or aromatic side chains. This follows from the notion that the subunit interfaces

are strongly hydrophobic as the trimer dissociates only in the presence of denaturants and its subunits are not covalently joined¹².

Trimeric haemerythrin is a compact structure maintained by strong helix-helix interactions between subunits (Fig. 2). The long axes of the subunits are tilted about 40° from the molecular 3-fold axis in a right-twisted manner, such that subunits cross at an angle of approximately 68°. The principal interactions occur where the B helix of one subunit crosses the A' (93°) and B' (-84°) helices of another subunit. These interactions are rather tight; crossed B and A' helix axes are separated by < 8 Å, in a manner reminiscent of the B-E helix cross observed in myoglobin, where two glycine residues are close to each other²⁶. A less extensive perpendicular contact also exists between C and B' helices (94°). There may be 3-fold axial interactions between residues 20 of the A helix and between residues 49 of the B helix. In addition, there may be interactions between the B helix and residues 9-11 of a neighbouring N-terminal arm. Otherwise, non-helical regions of the molecule are not involved in maintaining the trimeric aggregate.

Structures are known for other helix-bundle proteins which aggregate primarily through helix contacts. In these cases the associations continue the antiparallel helix-bundle motif with extensive helix contacts between each pair of interacting subunits²⁷. This antiparallel mode of association is observed in the 2-fold interaction of cytochrome *c'* (ref. 7), in the principal interaction of the helix-bundle of apoferritin⁵, and in the equatorial interaction of the tobacco mosaic virus helix²⁸ or disk⁴. While one of the subunit interfaces in octameric haemerythrin does involve helix-helix contacts (these in the perpendicular mode of association, contrary to statements in ref. 27), the non-helical N-terminal arm also contributes extensively to this interface and we do not consider this to be an association of helix bundles. The exclusively perpendicular mode of association in trimeric haemerythrin is fundamentally different from the other helix-bundle associations, and can be viewed schematically as the crossing of two helix hairpins (B-C and A'-B' faces of neighbouring subunits). The marked twist of the helix-bundle determines that only three intersubunit helix contacts can occur at each hairpin cross, and these are observed between the B and A', B and B', and C and B' helices of trimeric haemerythrin (if the helix bundles were of opposite twist, four such intersubunit contacts could form). The perpendicular mode of association thus involves inherently less extensive contact between each pair of subunits than the antiparallel mode. However, while it is well suited to dimer formation, the antiparallel mode of association is geometrically unfavourable for 3-fold aggregation. Instead, three mutually perpendicular B helices form the nucleus of a tight trimeric association in which two faces of each subunit interact in a cyclical manner with the two neighbouring subunits. In view of this diversity in mode of association adapted to molecular symmetry, we await with interest the further determination of structures of multimeric helix-bundle proteins²⁹.

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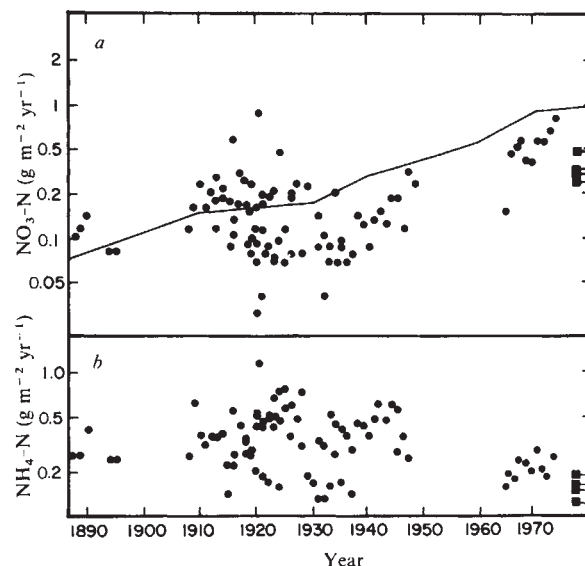
Corrigendum

Historical evidence for a dramatic increase in the nitrate component of acid rain

P. Brimblecombe & D. H. Stedman

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The increase in nitrate deposition in the northeastern USA was somewhat exaggerated in Fig. 2 of our recent paper¹. A replotted version is shown below. The data from 1964-74 are total deposition data (wet and dry) for Hubbard Brook². The 1976-79 data shown as squares are wet deposition only from Wilson *et al.*³. It now seems that both European and US nitrate deposition have risen by about a factor of 5 over the past century. The nitrate flux alone appears to exceed the neutralizing ability of the ammonium flux, independent of any SO₂⁻ contribution. This conclusion is reinforced for the USA by Miller⁴ who states that "ambient levels of NO₂ increased a total of 20% between 1970 and 1980".



We thank Dr J. A. Logan for drawing our attention to the error.

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Nuclear power: the politics of protest

Philip Gummert & Wolfgang Rudig

Rationality and Ritual: The Windscale Inquiry and Nuclear Decisions in Britain.

By Brian Wynne.

British Society for the History of Science: 1982. Pp.222. Pbk £6.50.

Anti-nuclear Protest: The Opposition to Nuclear Energy in France.

By Alain Touraine *et al.*

Cambridge University Press: 1983. Pp.200. £19.50, \$34.50.

HOW FAR is it possible to make nuclear power policy by reason instead of by fighting over it? And what is the political significance of the anti-nuclear movement? These questions are far from academic, as recent history shows. Anti-nuclear activity has, on occasion, led to bloodshed, and the protest movement has generated critiques of nuclear decision-making as dominated by technocratic elites which are not subject to democratic control, and as symptomatic of a pathological crisis in industrial society.

Different methods have been tried to contain the protest. Public information programmes and elaborate formal inquiry processes now operate in several countries. But such involvement in the decision-making process as these measures allow inevitably falls far short of what many desire. Moreover, it does not always even extend as far as it may at first seem.

This point is well illustrated in Brian Wynne's book. While riots were breaking out at nuclear sites in France, British opposition in 1977 to the construction of a thermal oxide reprocessing plant (THORP) at Windscale was channelled into a major public inquiry which lasted 100 days, took evidence from 146 witnesses and received some 1,500 documents in evidence. Yet, argues Wynne, the "rationality" of the process was only apparent, not real; the process was a ritual, not a conspiracy but a collective self-delusion about the possibility of free political control over a decision which, in reality, was simply the latest step in a long process of policy implementation. Moreover, he

argues that the use of ritual as a means of both handling complexity and maintaining the myth of the rational control of technology is not an exclusively British political device:

What connects such apparently dissimilar episodes as the Windscale decision, the Gorleben decision, and the official reaction to Harrisburg, is the exorcism of any sense of ignorance and lack of control. These features threaten authority, so they are wrapped in the ritual of political language and participation. Windscale affected discovery of a factual answer to a decision which really concerned complex political choice; although it apparently acceded to public hostility, Gorleben implicitly measured this against a yardstick of 'fact' erected by the expert authorities, rather than as an expression of public choice; the industry's reaction to Harrisburg was to argue that such an accident was in principle foreseeable and thus eradicable [p.6].

The theme of replacing political choice with a "factual discovery process" in which an inspector — typically a judge — presents his "findings" in a manner which, by analogy with the presentation of scientific findings, carries great authority, recurs throughout the book.

In addition to this analysis, the main strength of Wynne's book (offsetting rather dense language and some minor factual errors in the historical and theoretical chapters) is its richly detailed account of the Inquiry itself. As a lay advocate for one of the objector groups, Wynne is an excellent guide to the pressures under which the objectors worked — the uncertainty about whether there would be an

inquiry, then the doubts as to what it would cover, the problem of raising money to prepare and fight a case (including here the huge difficulty of trying to orchestrate the objectors' cases), and finally the exhausting task of actually participating in the inquiry. Even if some of the details of people and organizations may be a little lost on non-British readers, it is all splendidly told.

Far, then, from showing how Britain makes its nuclear decisions more democratically than others (as some newspapers claimed at the time), the Windscale Inquiry shows instead the difficulty of mounting serious opposition when policy has gained such momentum and when the imbalance of resources is so great. On the positive side, however, the volume of information released during the Inquiry does at least allow the possibility of holding the nuclear authorities more accountable in future for their actions. And, of course, the British inquiry process acts as a politically expedient outlet for protest, an outlet which has not been available in France where anti-nuclear groups have not only turned to direct action and civil disobedience but have also developed a range of radical challenges to the political system.

These differences in political culture emerge clearly from *Anti-nuclear Protest*, both in its content and style of presentation. Written by Alain Touraine, one of France's leading sociologists, and his research team, it is *not* a traditional academic study of the French anti-nuclear movement as the title would suggest. (For such accounts one might turn to N.J.D. Lucas' *Energy in France* (Europa Publications, 1979) or D. Nelkin and M. Pollak's *The Atom Besieged* (MIT Press, 1981).) Instead, Touraine *et al.* attempt to clarify and contribute to the ideological self-understanding of that movement, seeing their role in direct intervention in the movement's development. To this end, they set up a sequence of group discussions in which anti-nuclear activists of various complexions either discussed with each other or were confronted by other participants in the nuclear conflict — nuclear technocrats, communist trade unionists,

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Contrast in styles: Mr Justice Parker (on the right of the picture), inspector for the Windscale Inquiry, visits the control room of the plant. But was it just a ritual? Right: anti-nuclear protestors in France.

ecologists' leaders — and by the views of the authors themselves on the stances the movement should take.

After a brief introduction to the basic features of the French nuclear programme and the history of anti-nuclear action up to 1979, the book plunges into the arguments of the group meetings. The reports of these discussions give the reader a direct account of the points of view and styles of thought of some French anti-nuclear circles. Herein lies one of the values of the book, although the details of who said what are often rather lengthy and repetitive, and there must be considerable uncertainty as to how typical the opinions expressed are of the movement as a whole.

The authors' intention is, however, not just to document the various views held by French nuclear activists. Their main aim is to outline Touraine's vision of the anti-nuclear movement's potential social role and to report the history of his attempt to convert the activists to that view. For Touraine, a social movement first has to identify its adversary; this he sees not in industrial society, capitalism or the nuclear industry as such, but in "technocratic power". Secondly, the movement has to recognize the "stakes" of the struggle. Here, Touraine rejects the fundamental opposition expressed by many anti-nuclear protestors and suggests that they should formulate concrete "counter projects" such as alternative forms of energy production with a positive effect on employment. In short, Touraine and his colleagues want to lead the anti-nuclear groups to more pragmatic forms of political action. At the same time they perceive the groups as the central agent of social change and modernization. In opposing "technocracy" they lead the way towards the "post industrial society". In the end, though, the authors succeeded in converting only a limited number of activists to their position.

Finally, what contribution does *Anti-nuclear Protest* make to our understanding of the nuclear issue? The authors' approach leaves many questions open. There is, for example, no convincing attempt to show where the political support for a transition to a post-industrial society might come from. Moreover, the book's account ends in 1979, contains no new material or updating, and is in certain respects already overtaken by events. Nevertheless, Touraine does a service by extending our conception of the anti-nuclear movement beyond the traditional one of a single-issue pressure group. By representing anti-nuclear protest as part of a wider process of potential political change, this book, like Wynne's, raises questions far beyond its specific subject matter and will be an important reference point for future work in the area. □

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Talking patterns

Tim White

The Myths of Human Evolution.

By Niles Eldredge and Ian Tattersall.
Columbia University Press: 1982. Pp. 196.
£15, \$22.50.

AN INSPIRATIONAL 1972 paper by Niles Eldredge and Stephen Gould has provoked fruitful empirical work and active debate about whether evolution proceeds mostly by phyletic gradualism or by punctuated equilibrium (Stanley's "rectangular" evolution). Characterization of evolution within the hominid clade was traditionally based on assumed gradualism and some anthropologists still dogmatically defend pan-gradualism among hominids. Eldredge now joins Ian Tattersall, an anthropologist at the American Museum, to counter these Darwinian gradualist young and old fogeys in a new book on human evolution. The authors undertake their mission with revolutionary fervour, arguing rectangular evolution for the hominids and their culture, even insisting that we must grasp the episodic nature of change in order to understand where our species is going.

The first four chapters outline the development of gradualism and the case against it. This third of the book is lucid and literate, reading so smoothly that one may slip past unsupported or overstated arguments such as "... speciation is what triggers all but the most trivial examples of adaptive change" (p.62). The authors warn: "... reductionism pervades the minds of most active scientists today" (p.13). They also dislike creationism, sociobiology ("biological determinists") and myths. Despite some overdone dialectic — "The myth that change itself produces new species is gone. Instead it is new species that produce change" (p.61) — much of this section is enjoyable and I even agreed with parts of it.

Chapters 5 and 6 comprise the hominid half of the book. They differ little from sections of introductory anthropology textbooks except where the authors offer revisionist historical accounts of discovery and interpretation of the hominid fossils. For example, we are told that Louis Leakey "quickly swallowed his disappointment" when Olduvai Hominid 5 didn't substantiate his preconceptions about early true man in eastern Africa. In fact, Leakey was so convinced that this fossil was his pre-conceived true man that he proposed the outrageous name *Titanohomo mirabilis* for it before formally naming it *Zinjanthropus* and describing it as man's direct ancestor!

Eldredge and Tattersall actively perpetuate some myths in their effort to promote punctation. For instance, the authors note episodic change in the "sudden" appearance of Oldowan stone tools. How one wonders, could the first

stone tools gradually record themselves? The authors are also content to invoke Mary Leakey's myth that the Oldowan tool "kit" consisted of a wide variety of functionally distinct tools used by food-sharing hominids occupying shelters they constructed at home bases. Binford's appropriately entitled *Bones: Ancient Men and Modern Myths* has done much to eradicate such tales.

In summing-up Eldredge and Tattersall claim that they really did look for gradualism but just couldn't find it. They announce that they have "debunked" and "skewered" the myths of gradual biological evolution and cultural development, concluding: "The patterns, in other words, speak for themselves. They are there for all to see. We have not simply invented them to fit a set of preconceived notions" (p.176). I remain sceptical of this but satisfied that the authors could make radioactive decay appear punctuated — all you have to do is look closely enough.

It is not clear who the book was written for. There is no preface. If intended for the popular audience, why the detail? I stopped on p.144 to note all cranial capacities, dates and specimen numbers — the results: 800; 1,000; 1,000; 2,000; 1.7; 800; 700,000; 450,000; 900; 1,200; 1.6; 0.4; 0.5; 1.2; 3,733; 900; 1,200; 1.2; 1,100. Yet, amazingly, the book is devoid of references and lacks a bibliography. For these reasons it misses popular, student and professional audiences. This is sad because there are some good things in here.

Lennon and McCartney wrote: "You tell me that it's evolution — well you know, we all want to change the world". Eldredge and Gould were revolutionary in insisting that palaeontologists should treat stasis as data but we cannot change the world of the past to fit our preconceived notions no matter how much we might want to. The history of the hominid clade simply lacks adequate documentation to choose between gradualist or punctuated alternatives on most scales. Evolution on the molecular level clearly proceeded both ways. There is good palaeontological reason to suspect that the rectangular model best accommodates Pliocene and early Pleistocene hominid evolution. On the other hand, Cann's recent analysis of the human mitochondrial genome suggests that anatomically modern humans could not have arisen in a recent speciation event. Could feedback between complex social interaction, technology and Pleistocene hominid morphology have provided the gears for the motor of natural selection to turn in producing one of the few cases of Darwinian gradualism on a geological time scale? It is too early for definitive answers just yet, but it is worth remembering that Homer's myth did become Schliemann's Troy. □

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What has happened in biotechnology

E.C. Dart & A.J. Coleman

Biotechnology: A Comprehensive Treatise in 8 Volumes. Vol. 1 Microbial Fundamentals; Vol. 3 Microbial Products, Biomass and Primary Products.

Edited by H.-J. Rehm and G. Reed.
Verlag Chemie: 1982/1983. Each volume pp.520, \$309, £130.

MOST industrial folk would agree that biotechnology is likely to have its greatest impact in converting low-grade natural products, such as cellulose, into materials of significantly higher added value. Implicit in this view is the recognition that fermentation processes are generally inefficient and that biology is best used to generate molecules of great complexity. Such messages have clearly been taken on board by the publishing fraternity who have something to teach us in the way they carry out complex cellulose conversions. Each volume of this proposed eight-volume series on biotechnology contains about 1.5 kg of cellulose and sells at £130. Its bulk price therefore comes out at about £87,000 per metric tonne, not a bad mark up by any biotechnological (or literary) standards.

What are we offered for this investment? The editors have gathered together a distinguished editorial board to oversee the preparation of the series, the objective of which is to provide researchers and teachers alike with a comprehensive bio-

technological reference work. Volumes 1 and 2 aim to set the scene by providing the microbial and engineering fundamentals, while subsequent volumes will deal with the usual range of products and product areas.

Sadly, a scan of the proposed product coverage (to date only Vols 1 and 3 are available) suggests some notable omissions. The list of complex compounds and polymers, for example, does not include polypeptides and proteins. Monoclonal antibodies and vaccines are nowhere to be seen. Are we to conclude — as one does from a more detailed inspection of Vols 1 and 3 — that we have here more a description of the biotechnology of times gone by, important though some of this may be? Surely it is the new biology of cell, gene and antibody cloning that has given biotechnology its uplift and while some of its undoubted promise will take longer to realize than most of the pundits would have us believe, the new and the old need to be put into a more believable perspective than we are given here. The chapter on genetic engineering, for example, does scant justice to a most exciting technology. We are not brought up to date with its scientific achievements nor are we made aware of its present limitations. Similarly the chapter on mutations does not even mention site-directed mutagenesis which will surely become more important as more key synthetic and regulatory genes are cloned.

We are promised critical assessments of industrial applications yet there is little evidence of this. For instance, in Vol. 3 human food from algae is mentioned as a challenge to the industry with little or no guidance as to what problems need to be addressed to bring this to reality. There is a description of single-cell protein pro-

duction from alkanes yet little mention of why it is not now being exploited vigorously. Industrial acetic acid fermentation processes are discussed, but there is no comparison of their economics with those of production methods based on petrochemicals.

As with many other multi-author volumes, the standards of individual chapters show great variation. Some are so compressed as to be almost not worth publishing. Others, such as the chapter on ethanol from biomass, are well written but need the space they are given to do justice to their subject (the ethanol chapter takes up a quarter of Vol. 3). This section, along with those on amino acids and polysaccharides are the highlights of Vol. 3. Adequate space is also devoted to an article in Vol. 1 on sources of microorganisms and we are rewarded with a most comprehensive and readable account of the subject. For future volumes the editors must encourage the inclusion of more up-to-date material and a more detailed coverage of those areas they judge to be important. Otherwise this background work will only fill a few background shelves in the more affluent libraries. □

Ed Dart is Head of ICI's Corporate Bioscience and Colloid Laboratory, Runcorn, Cheshire. Alan Coleman is on secondment from ICI to the Department of Industry's Biotechnology Group.

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Biology by numbers

S.D. Dover

Computing in Biological Science.

Edited by M.J. Geisow and A.N. Barrett.
Elsevier Biomedical: 1983. Pp.445.
Dfl.160, \$65.

THE best description of this book is that it would not be out of place as one volume in an annual series. The articles are interesting and thorough, but they are with one or two exceptions descriptions of research projects as they have been conducted by particular groups. In this sense the book is not a collection of review articles. Nor does it attempt, in the editors' own submission, to be complete, but rather to be illustrative of what can be and has been done.

Despite these criticisms the structure and quality of the articles make it a very readable book for browsing and picking up ideas. Many of the computational techniques are going to be applicable in a number of areas of biology and their methods and use are made very clear.

The editors have divided their subject matter into four sections — modelling, image analysis, numerical analysis, and microprocessors. The last two sections cover well established specialist areas. Unlike the other, major sections, however, these have not been described in ways which bring out the particular importance to any one part of biology. Modelling has been broken down into subsections which deal with diverse aspects such as the modelling of cells and cell components, and the building of molecular models of macromolecules. Similarly, the section on image analysis covers the processing of electrophoretic gel pictures and electron microscope three-dimensional image reconstruction.

In conclusion I am faced with a contradiction. There is no one topic for which I could justify purchasing this book, but I shall use it often, particularly for final-year undergraduate projects and for its useful examples. □

S.D. Dover is a Lecturer in the Department of Biophysics, Kings College, University of London.

Access to the Sun

Robert Rosner

The Sun as a Star. NASA SP-450.

Edited by Stuart Jordan.

NASA/CNRS: 1982. Pp.518. Pbk \$11.

ALTHOUGH several good monographs on solar physics have recently appeared — F.Q. Orrall's *Solar Active Regions* (Colorado Associated University Press, 1981) and E.R. Priest's *Solar Flare Magnetohydrodynamics* (Gordon & Breach, 1982), for example — these have tended towards treatment of specialized topics and an up-to-date overview has been lacking. *The Sun as a Star* does a commendable job of filling this gap, reviewing much of current observational and theoretical work relevant to present-day solar research, and its possible extension to stellar research. The material is, in large part, written at a level appropriate to first-year graduate students; it is by far the most accessible general account of solar physics I have seen to date, and has already been extensively used in our solar and stellar physics course.

The chapters have each been written by active researchers, and generally convey an accurate sense of the broad thinking on the subject; there is relatively little of the narrowness of perspective one at times encounters in similar reviews. Particularly noteworthy and complete are the discussions of photospheric phenomena and global oscillations by J. Beckers and F.-L. Deubner, of wave generation and propagation by R. Stein and J. Leibacher, and of magnetic flux tubes by H. Spruit. These contributions bring together material which has not been recently reviewed elsewhere in such depth, and do so at a level which is comprehensible to the neophyte.

I also very much enjoyed the multifaceted discussions of plasma heating in the solar outer atmosphere given in individual contributions by G. Withbroe, D. Wentzel, J. Hollweg and R. Kopp. Indeed, the entire volume is characterized by clear organization and careful integration of the various subject areas; most chapters have an accessible, didactic style, which is most welcome for a book intended to be an introduction to the subject (and is a surprising feature for a work written by such a wide variety of specialists).

Only a few topics are treated at a level dissonant with the rest of the volume. These include, principally, the (theoretical) discussions of solar flares, which are not fully self-contained but rather require considerable familiarity with the plasma physics literature in order to be properly appreciated (the text edited by E.R. Priest, mentioned above, as well as the review monograph from the Skylab Solar Flare Workshop edited by P.A. Sturrock (Colorado Associated University Press, 1980) would provide some of the necessary

background). An annoying quirk is the placement of the table of contents some 53 pages into the text following the preface and summary (both given in English as well as French); thus, it is somewhat difficult to find the table of contents itself, as well as to locate specific articles.

Several useful features grace the text, including the summary given in the beginning by S. Jordan (which provides a "road map" for the remainder of the volume), and the final chapter (by R. Rutten and L. Cram), in which possible future research directions are outlined. The coverage of the literature is more than

adequate, and includes lists of spectral atlases and the like. On the whole, the editor has assembled a monograph of great value to the budding solar astronomer: it is an ideal text for an introductory course in solar and stellar astronomy (even its price is very modest in comparison to standard monographs in astronomy), and provides an adequate and well-referenced overview for all astronomers interested in solar phenomena and its analogues in astrophysics. □

Robert Rosner is Assistant Professor of Astronomy at Harvard University.

Changeable cells

David M. Prescott

Cell Differentiation: Molecular Basis and Problems.

Edited by Lutz Nover, Martin Luckner and Benno Parthier.

Springer-Verlag: 1982. Pp.650. DM118, \$55.

The prime object in the study of cell differentiation is that of understanding the mechanisms of gene expression and their regulation. The complexity of these mechanisms is exemplified by the fact that probably no other phenomenon in biology draws so heavily upon so many research areas, including molecular genetics, immunology, mechanism of hormone action, cell reproduction, tumour biology, virology and others. The contributors to *Cell Differentiation* take a correspondingly broad approach to the subject, exploiting what has been learned from a wide variety of experimental systems to assess where we stand.

The book is divided into three parts. The first deals with general molecular genetics of prokaryotes and eukaryotes — for many readers this is all review, but it is well done and should be valuable for even advanced students — whilst Part II contains a detailed discussion of the principles of gene expression and its regulation as these principles have developed from the study of many kinds of prokaryotes and eukaryotes.

Both of these sections are up-to-date, comprehensive and well referenced. There are a few gaps, however. Almost nothing is said about studies on differential transcription of mRNA during development, abundance classes of mRNAs and differences in mRNA populations among different tissues. Some readers may be unhappy about the absence of information on patterns of repeated and unique sequences and their possible significance in gene function. No mention is made of the Britten-Davidson model of regulation of expression. Some might also object to the broad definition of cell differentiation as virtually any change in gene expression.

For the authors, differentiation may be reversible or irreversible, includes the phenomenon of "determination" during development, and applies to substrate-mediated regulation of gene expression in prokaryotes.

The final section consists of in-depth descriptions and analyses of a wide range of experimental systems that bear on gene expression or cell differentiation. These include contributions on the arabinose genes in *E. coli*, chloroplast differentiation, insulin action, isozymes, storage proteins in plant seeds, the immune system, bacteriophage cycles, morphogenesis of *Acetabularia*, development of *Dictyostelium*, plant crown gall tumours, oncogenesis in animals, the cell reproductive cycle, polytene chromosomes and several other topics. Some of these chapters are more thorough than others, but all are worth reading. The main criticism is the omission of references to some important research publications. The reader could also immediately think of other analyses or experimental systems that perhaps should have been included. For example, almost nothing about the rapidly developing field of developmental genetics appears in the book. One might also have wished for a short final chapter to summarize the principal conclusions and insights gained from various experimental systems, and to formulate an outline of what remains to be done.

It is easy to criticize books on cell differentiation and development because the problem has so many intricacies — everyone has his own ideas about where and how the solution should be sought. The editors and authors of this book have theirs. Overall, this is nonetheless a valuable book; it is well organized and clear, and contains an enormous amount of accurate information about a wide diversity of organisms. Although the book deals with research areas that are moving ahead rapidly, it should for many years provide both students and researchers with a rich source of basic information. □

David M. Prescott is Distinguished Professor in the Department of Molecular, Cellular and Developmental Biology, University of Colorado.

Earth Sciences

- AKIMOTO, S. and MANGHNANI, M.H. (eds). High-Pressure Research in Geophysics. Advances in Earth and Planetary Sciences, Vol. 12. Pp.632. ISBN 90-277-14398. (Reidel: 1982.) Np.
- BARRER, R.M. Hydrothermal Chemistry of Zeolites. Pp.360. ISBN 0-12-079360-1. (Academic: 1982.) £31, \$57.50.
- BARRY, R.G. and CHORLEY, R.J. Atmosphere, Weather and Climate, 4th Edn. Pp.407. Hbk ISBN 0-416-33690-6; pbk ISBN 0-416-33690-6. (Methuen: 1982.) Hbk £14.50; pbk £6.50.
- DACKOMBE, R.V. and GARDINER, V. Geomorphological Field Manual. Pp.254. Hbk ISBN 0-04-551061-X; pbk 0-04-551062-8. (George Allen & Unwin: 1982.) Hbk £16; pbk £7.95.
- DAWES, P.R. and KERR, J.W. (eds). Nares Strait and the Drift of Greenland: A Conflict in Plate Tectonics. Pp.392. ISBN 87-17-02969-4. (Meddelelser om Grønland: 1982.) Np.
- DENNIS, J.G. (ed.) Orogeny. Benchmark Papers in Geology/62. Pp.379. ISBN 0-87933-394-4. (Hutchinson Ross: 1982.) \$46.
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Monodisperse polymer particles — a step forward for chromatography

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THE development of a process to produce single-sized spherical particles for use in chromatography columns has led to a dramatic increase in speed and resolution achieved with liquid chromatography. Traditionally it has only been possible to produce monosized particles of less than 2 μm in diameter. Only an average size could be guaranteed for larger particles, so that commercial packing material with an average size of, say, 10 μm would consist of a range of particles both considerably larger and considerably smaller than 10 μm . When packed in a column, the small particles would then block up the channels between the larger particles giving a low flow rate for a particular mobile-phase pressure. This would also lead to a variation in the resistance to fluid flow across the column, which in turn would produce band-broadening, giving poor resolution.

Using a column packed with the new monosized particles with the same average size, the channels between the particles remain free. Therefore the flow rate is higher, the resistance remains the same across the column, and the resolution is increased.

Since it is clearly advantageous to use columns packed with particles of just one size, or monodisperse polymer particles, why is it that they have only just become available? To answer that it is necessary to look at the techniques traditionally used to produce commercial packing materials.

Traditional techniques

Ion exchange macroporous polymer particles for use in chromatographic separations are normally produced by the suspension polymerization of styrene and divinylbenzene in the presence of an inert liquid. This mixture, with an oil soluble initiator, is dispersed in water by mechanical forces in the presence of a dispersing agent for stabilizing the droplets. Polymerization takes place on heating. Since one monomer is bifunctional, clusters of precipitated cross-linked polymer are formed inside the droplets.

After polymerization, the inert liquid is removed, leaving a macroporous structure, consisting of spherical particles built up of a solid matrix with relatively large pores.

The particles may then be transformed to ionic exchange resins by the introduction of anionic or cationic groups on the benzene rings.

The fact that the droplets are formed by mechanical forces means that the particles produced cover a broad range of sizes. Normally a fractionation has to be carried out to achieve a more narrow size distribution. However, such processes are tedious and relatively ineffective.

Producing monodisperse particles

Recently, Ugelstad¹⁻³ developed methods for producing highly monodisperse systems — systems with particles of exactly the same size — of between 1 and 100 μm in diameter. His methods of preparation may be used with several types of monomers and are particularly suitable for producing macroporous particles, see Fig. 1. Ugelstad's basic 'prototype' particles can be changed by a multi-step modification to give three types of hydrophilic ionic ex-

change systems, the so-called Monobeads. These are Mono Q, an anionic exchanger, Mono S, a cationic exchanger, and Mono P, a polybuffer exchanger. Together they form a system for the separation of biomolecules⁴⁻⁹ giving resolutions comparable with those achieved using electrophoretic techniques. Examples of applications are: the analysis and purification of human serum proteins¹⁰⁻¹², the separation of peptides^{13,14}, the analysis of urine protein composition¹⁵ and the purification of surface proteins from bovine viral diarrhoea virus¹⁶. The separation time is considerably less than that required with conventional systems and may be reduced by as much as 240 times, as shown in Fig. 2.

Other applications

Not only do the new media improve the chromatographic techniques already in use, they also extend the application of chromatography to include separations which have so far been impossible. These new applications include fast finger-

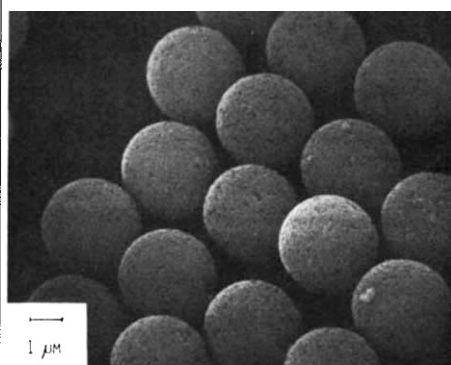


Fig. 1a 10 μm macroporous particles

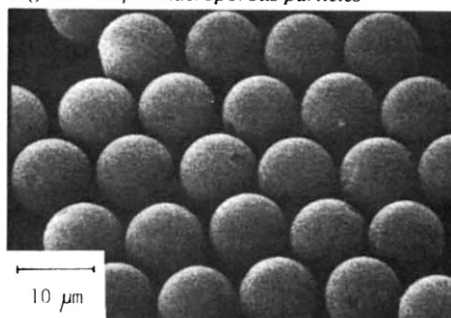


Fig. 1b 3 μm monodisperse magnetic particles containing 35% magnetite

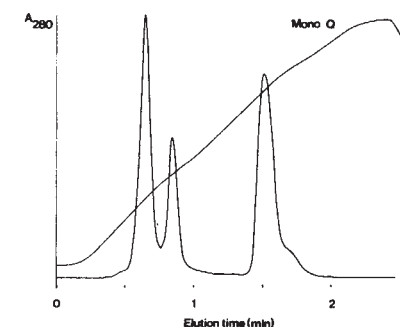
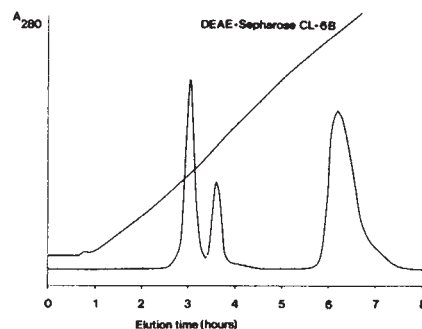


Fig. 2 Comparison of Mono Q with DEAE Sepharose CL-6B. A comparable resolution is achieved 240 times faster with Mono Q.

printing of biological samples for taxonomy, the production of delicate proteins such as interferon, the monitoring of industrial fermentation, as well as forensic applications and the monitoring of medical patients by the analysis of body fluids.

Monodisperse particles have recently been accepted as calibration standards for blood cell counters by the EEC Commission's Bureau of Standards. Furthermore, with the incorporation of up to 40% magnetite into the monodisperse particles¹⁷ they have been used for cell separation¹⁸, including the separation of cancerous from normal cells.

By adding those mouse monoclonal antibodies which bind specifically with cancer cells to bone marrow containing cancerous neuroblastoma cells, and then introducing 3 μ m porous magnetic particles coated with the goat-anti-mouse immunoglobulin which selectively binds to the neuroblastoma cells via the mouse monoclonal antibodies, and then passing the mixture through a magnetic field, Kemshead was able to trap the cancer cells without interfering with the normal cells. It is now planned to use this method in autologous transplantations. Fig. 1b shows the 3 μ m porous particles with 35% magnetite used in cell separation.

A final application for the monodisperse polymer particles is in immunoassay techniques²⁰⁻²² and special particles containing radioactive tracers have been used for blood circulation studies.

The industrial production of monodisperse polymer particles has been started at Dyno Industrier A.S. Norway.

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Chromatography and electrophoresis equipment

● A new line of gas filters and purifiers for use in gas chromatography has been announced by Labclear. New materials, such as Teflon O-rings, have been used to allow Labclear filters to be introduced with electron-capture detectors. No plastics or silicon rubbers, which cause noise problems on electron capture detectors, have been used. The filters and purifiers are equipped with nickel-plated fittings which avoid any galling of threads after repeated use.

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● Whatman Chemical Separation has recently introduced Prep 25, a production scale chromatography column designed for the ion exchange separation of biopolymers. Prep 25 features a 25 litre column bed capable of absorbing up to 2 kg of biopolymer. Operated at 5 to 6 p.s.i. (0.4 bar) Prep 25 gives flow rates of between 0.8 and 1.5 litres per minute. Whatman has also made available a stainless steel column for applications requiring steam sterilization.

Circle No. 101 on Reader Service Card

● Labquip have produced a new piece of submerged agarose gel electrophoresis apparatus which allows gels to be cast directly *in situ* with either a 8 or an 16 tooth comb, thereby catering for volumes from 1 to 40 μ l. Since the base of the tray is transparent to UV the gels may be viewed during the course of the electrophoresis. The lane positions are numbered, and a scale assists in determining mobilities. The addition of a levelling bubble helps in the uniform casting and running of gels.

Circle No. 102 on Reader Service Card

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



Labclear's gas filters and purifiers

● A new version of the 725 AutoInjector from Micromeritics can derivatize and inject liquid chromatography samples to allow unattended analyses of up to 32 samples. The new unit is called the model 725D AutoInjector. The dual sample AutoInjector can be used with an HPLC system.

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● The new isoelectric focusing (IEF) chamber from FMC Corporation's Marine Colloids Division, is a horizontal multi-purpose chamber. The IEF electrode frame is fully adjustable, making it possible to focus across either the width or the length of the plate. Cuing levers allow the operator to adjust the distance between the electrodes while the IEF frame is suspended above the gel, in order to line up the electrodes with wicks. A window in the centre of the IEF frame allows the operator to remove the sample applicator mask during the run without disturbing the electrodes. Submerged electrodes can be positioned at varying distances from the wicks to minimize the effects of regional buffer depletion.

Circle No. 104 on Reader Service Card

● Gilson has announced a second addition to its series of microprocessor controlled fraction collectors for liquid chromatography and HPLC. The new model 201-B includes several new features, such as an advanced peak detection circuit which combines slope sensitivity with level sensing; a new time-programmable mode which allows entry of peak, drop, or time collection within the specified window; and a new multicycle mode. The 201-B may be stacked in a Gilson HPLC system or used in configuration with other HPLC apparatus. The 201-B may be interfaced to laboratory computers, system controllers and automatic sample injectors.

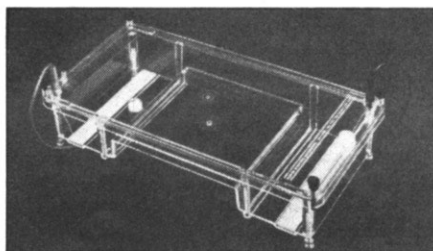
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The 725 AutoInjector from Micromeritics



The Atto fraction collector



The Hoefer HE99



Atto's slab gel chamber

Systems for chromatography and electrophoresis

● The new model 5500 liquid chromatograph from Varian Associates features fully-integrated control, microstep pumping, programmable detection with scanning, and structured upgradable software. A CRT and keyboard control instrument operation. The LC 5500's column/detector compartment is designed to accommodate built-in detectors (up to two at a time). For automatic operation, an autosampler can be added, and methods set up in the 5500 will control automatic injection of up to 60 samples. The model 5500 interfaces with the new Vista 402 chromatography data system or with an external computer.

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● A new line of electrophoresis equipment has been introduced by Jordan Scientific with models for both polyacrylamide and agarose gel systems, including units for vertical slab gel and submarine gel electrophoresis. Model JVA-180 is an adjustable vertical slab unit for gels up to 15 cm wide and 15 to 43 cm tall. Model JSB-75 affords quick and easy preparation of submarine gels 12.5 cm wide by 19 cm long. Other models available include a double slab vertical gel unit, units for nucleic acid sequencing, and both micro- and macro-sized submarine gel units. A selection of combs and spacers is available for all units.

Circle No. 107 on Reader Service Card

● Biotech Research Laboratories have introduced a new horizontal system for submerged gel electrophoresis. The unit can be used in routine analysis of DNA samples such as in assessing the purity of plasmid DNA, the extent of restriction enzyme digestion and for the initial analysis of restriction enzyme digestion. Gels of 2 × 3 inches can be cast in a pouring and carrying tray or on slides. Pre-formed gels can be stored for at least 2 weeks in electrophoresis buffer or distilled water as required.

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● The Hoefer HE99 is a horizontal unit (33½ × 18 × 7 cm) for agarose gel electrophoresis. Two buffer chambers with platinum wire electrodes are separated by a raised platform which supports a submarine or a bridge gel. The unit comes with a casting tray 15 cm wide and either 10, 15, 20 or 25 cm long. Each tray is fitted with a UV-transparent running plate. Bridge gels, 15 × 15 cm, are cast directly in the unit. The comb rests across the rim of the chamber at any position and can be adjusted in height to control the well depth. The entire unit is injection moulded of a clear, UV-transparent acrylic for monitoring nucleic acid separations. The electrical circuit is completed only when the safety lid is in place and the unit is fully enclosed.

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● Paragon is new agarose gel electrophoresis system that has been introduced by Beckman. The system includes a power supply, an incubator and oven, a wet processor and gel frame, an electrophoresis cell and a sample applicator. Also available are a series of reagent kits for serum proteins, for lactic dehydrogenase isoenzyme, for creatine kinase isoenzyme, and for haemoglobin and lipoproteins.

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● A new instrument for thin layer chromatography has been launched by Labor Mim — the Chrompres-10 — which will run 40 cm length preparations. Labor Mim also manufacture apparatus for electrophoresis and HPLC.

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● The range of electrophoresis systems available from Genetic Research Instrumentation includes a disc tube gel, a vertical slab gel chamber and preparation unit, and a horizontal slab gel chamber for isoelectric focusing, agarose and paper electrophoresis techniques.

Circle No. 112 on Reader Service Card

● The Atto range of column chromatography apparatus is now available from Genetic Research Instrumentation. The Atto system is based on the mini fraction collector, which has a capacity of 150 tubes, and drop time collection in the 0–40°C range. Two mini U/V monitors are available. The Atto Perista peristaltic pumps will drive the columns up to maximum pressure of 28 p.s.i. and will produce a pulseless flow.

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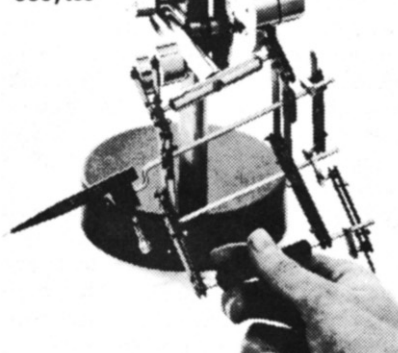
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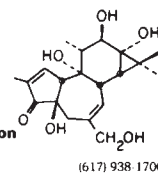
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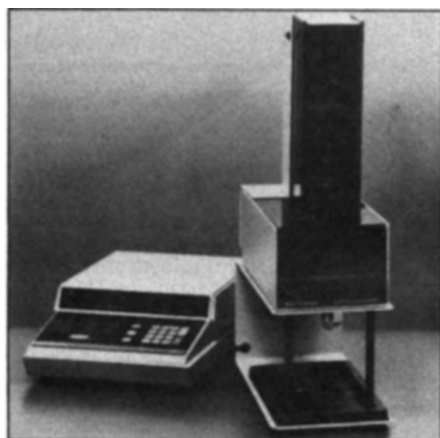
● Two new HPLC pumps, the MSI model 301 and the MSI model 310, have been introduced by Major Scientific Instruments. The model 301 is a high-volume (2–99 ml per minute), low-to-medium pressure (0–1,500 p.s.i.) pump. The model 310 is a small-volume (0.2–9.99 ml per minute), high-pressure (0–10,000 p.s.i.) pump.
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● A new HPLC ion exchange material, Spherisorb 5 SAX, has been launched by HPLC Technology which is designed to give efficiencies of up to 50,000 plates per metre. The acidic buffer solvents are kept at comparatively low pressures giving elution speeds of 1.0 ml per minute at 1,300 p.s.i. for 25 cm columns.
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● Du Pont has introduced a Zorbax phenyl column for HPLC with reversed phase selectivity for moderately polar compounds. The new phenyl column offers an alternative selectivity to other Zorbax alkyl group reversed phase packings. The columns have an internal diameter of 4.6 mm and are available in 15 cm and 25 cm lengths.
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● The model 1330 HPLC dual-piston reciprocating pump from Bio-Rad features a new electro-mechanical design which provides constant-flow operation between 0.1 and 10.0 ml per minute against back pressure of up to 6,000 p.s.i., and produces an almost pulse-free output.
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● Beckman Instruments' model 504 Autosampler automatically injects 102 samples into a column for HPLC analysis. The autosampler can inject samples from 1 to 100 μ l or inject 1 μ l from a sample of 10 and can store nine programs for automatic retrieval. Accuracy is 1% of syringe volume and repeatability is 0.5% of syringe volume. The model 504 will operate in a cold room.
Circle No. 118 on Reader Service Card.



Beckman's model 504 Autosampler

● Beckman's new series 341P HPLC system will separate biochemical macromolecules on both semi-preparative and analytical scales by size exclusion chromatography. The 411P is designed specifically for high performance gel filtration chromatography. The isocratic system can be used for work involving all types of proteins. The new system includes an isocratic liquid chromatograph, the model 160 selectable wavelength UV detector with 254 nm and 280 nm filters, a mercury lamp and a Beckman Spherogel TSK 3000 SW column.
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● A new reverse phase system chromatography (RPSC) column is available from Beckman which is designed to resolve large biopolymers. The column uses large-pore, spherical silica particles which are maximally bonded with a short chain. The 7.5 cm column length is intended to decrease separation times and to conserve solvent.
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Gels and packing materials

● Gelman Sciences now offers a specialized membrane for lipoprotein electrophoresis. Called Sepraphore-L, it is a microporous cellulose acetate membrane, that produces resolution of the major lipoprotein fractions, especially in the beta-pre-beta region.
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● Gradient Laboratories has introduced a new continuous, high concentration concave polyacrylamide gradient gel, with Bis as the cross linker. The GG-HX, or the highly cross-linked polyacrylamide gel, is specifically formulated for use in the resolution of peptides and low molecular weight proteins. This gel provides a tool for obtaining pure proteins, without subjecting them to denaturing by SDS electrophoresis.
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● Fractogel TSK, now available from BDH Chemicals, is a new series of porous, spherical gels with high solvent capacities which are mechanically and chemically stable. The matrix is constructed from hydrophilic vinyl polymers, the pores of which are surrounded by intertwined polymeric agglomerates which increase mechanical stability. The surfaces of the gel particles, including the internal surfaces, exhibit hydrophilic characteristics owing to the ether linkages and hydroxyl side groups on the polymer. However, biological materials dissolved in appropriate aqueous media are generally not adsorbed onto the matrix.
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● The Fluoro-Tec ratio filter fluorometer with digital readout has been introduced by American Research Products. Filters are also changed in a few seconds. The tube circuit has a decade switch with five steps of high voltage adjustment (300, 450, 600, 750, and 900 V).

Circle No. 121 on Reader Service Card.

● A new disposable filter designed for HPLC filtration has been introduced by Gelman Sciences. Gelman have also introduced a new fluoropolymer membrane which is both naturally hydrophilic for use with aqueous samples and chemically resistant, and is compatible with most HPLC solvents. The filter is available in 0.45 μ m pore size.

Circle No. 122 on Reader Service Card.

● Two UV detectors have been introduced by HPLC Technology. These are the SF 773, which has a 0.001 absorbance unit, range, and the SF 757, which has a maximum sensitivity range of 0.005 absorbance units. Also available is a range of 300Å pore size silicas to aid in the analysis of proteins and peptides.

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● AcrylAide cross-linker is a new comonomer from FMC Corporation which renders gels oven dryable, even at polyacrylamide concentrations above 20%. It is used instead of cross-linking agents such as bis-acrylamide (bis). Gels made with AcrylAide cross-linker on Gel-Bond PAG film perform similarly to those made with bis, but can be oven dried without using a vacuum slab dryer and cellophane. AcrylAide cross-linker also makes tube gels stronger, allowing them to be more easily manipulated without damage. Applications of the AcrylAide cross-linker include SDS, gradient and acid/urea gel electrophoresis. The linker is compatible with popular stains, including silver stains. The open side of gels dried on GelBond PAG film permits direct contact with X-ray film for autoradiography and fluorography.

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